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A multidisciplinary study of the scyphozoan jellyfishes of lower Chesapeake Bay, 1 April 1968 - 31 March 1971: Completion Report

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COMPLETION REPORT

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PROJECT TITLE A MULTIDISCIPLINARY STUDY OF THE SCYPHOZOAN JELLYFISHES OF LOWER CHESAPEAKE BAY

PERIOD COVERED 1 April 1968 - 31 March 1971

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INTRODUCTION AND SUMMARY

This document will constitute the completion report for contract No. 14-17-0007-961 (as amended) entitled "A Multidisciplinary Study of the Scyphozoan Jellyfishes of Lower Chesapeake Bay." The contract period covered a time-span of approximately three years. Two annual reports and one semi-annual report containing details of progress have been submitted prior to this completion report.

The first portion of this document will consist of a brief summary of accomplishments for the entire contract period. The second portion will contain a rather detailed statement of progress for the final year. This latter information has not been reported previously.

The work of this contract has been stated in terms of a large number of objectives, phases, or jobs during the past several years. As the work has progressed, several phases have been added, others dropped and in several cases, phases have been combined into single objectives. For convenience in reporting, all previous jobs, phases and objectives can be summarized under five activities - (1) ecological studies (2) taxonomic studies (3) development and biochemical studies (4) physiological studies and (5) mortality studies. This plan will be followed throughout the report although it will become clear that demarcation of subject areas is far from absolute.

I. Ecological Studies

The ecological studies can be described under five major objectives.

1) The distribution and seasonal setting patterns of the attached stages of Chrysaora quinquecirra.

This was one of the original objectives of the contract

and one that was approached from the beginning. This objective was met during the first two years of the program and phased out. The results can be summarized as follows: The open waters of the major rivers and the waters of the lower Bay proper have very few polyps and/or cysts as compared to the tributary creeks and smaller rivers with limited run-off. This distribution does not appear directly related to salinity or to a lack of suitable substrate in the open waters. Most of the polyps are found in salinities between 10 and 20‰. There are many square miles of open waters in the major rivers within these salinity limits. Furthermore, a large number of natural oyster bars, in addition to shell planted for oyster culture provide adequate substrate for setting of planulae and development of polyps.

The concentration of attached stages in the smaller rivers and tributary creeks has important and favorable implications for control, especially if control of the attached stage is sought.

2) The distributional and seasonal setting patterns of the attached stages of Cyanea capillata.

In contrast to the pattern described for Chrysaora, Cyanea polyps and/or cysts are generally found in the open waters of lower Chesapeake Bay or near the mouths of the major tributaries. Whatever limits the numbers of Chrysaora polyps in these waters does not seem to operate against Cyanea. Cyanea also differs from Chrysaora in that the Cyanea planula generally does not metamorphose directly into a polyp, as is the case in Chrysaora, but develops into a larval cyst which under different stimuli will develop into a polyp. This aspect will be treated in more detail in another section.

The seasonality of planula formation is markedly different

from the summer forms. Cyanea medusae mature in later winter and early spring and it is at this time that planula formation and setting occur.

3) The distribution and seasonal setting patterns of the attached stages of Aurelia aurita.

Of the many thousands of polyps that have been examined in the field, only a very few have proven to be those of Aurelia, far too few to account for the abundance of medusae in the lower Bay and tributaries. We assumed earlier in the study that the origin of Aurelia must be exogenous. Laboratory studies have indicated that planulae do develop on Aurelia and that these do set and metamorphose into polyps over a range of temperature and salinity conditions that are widely met within lower Chesapeake Bay. Field studies have also indicated that small medsoid stages are found well up the tributary creeks. We now assume that we do not find the polyp stages in nature not because of their absence but because of their mobility.

Their distribution pattern has not been worked out; however we do not believe that the importance of this information gap presently justifies the major effort that would be required to solve it.

4) Field distribution of medusae of the three major species.

The field distribution studies have had two primary objectives (1) to document the seasonal appearance and disappearance of the medusae of each species and (2) to document if possible the geographic variation in abundance and the year-to-year variation in numbers.

The first objective has been adequately met for all three species, although dates of appearance and disappearance will vary from year to year.

The second objective is much more difficult to attain. The patchiness of distribution, the great day-to-day variation in numbers, and the great variability in visibility from day to day create almost insurmontable sampling problems. Even so, certain generalizations do emerge. Chrysaora and Aurelia have been extremely abundant in the Virginia tributaries each of the three years. From our observations we are not aware of any obvious or major fluctuations in abundance from one year to the next. In the case of Cyanea, however, a major and obvious increase in abundance in the lower York River and in Mobjack Bay was documented for the winter and spring of 1971. This increase was so spectacular that the difficulties of sampling could not obscure it. We have no explanation at present for this dramatic change.

5) Predators of Polyps

During the first summer of the contract period (1969) a short term study of predators of Chrysaora polyps was conducted. A number of potential predators was tested, and several were found to feed avidly on polyps when they were offered in a confined situation. This information has been developed into a short manuscript and submitted to the National Marine Fisheries Service for clearance. As predator studies became a major portion of the Maryland Jellyfish Research program, this similar activity was not pursued further at VIMS.

II. Taxonomic Studies

The taxonomic studies were added by amendment to the original proposal when it became obvious that problems of identification of the attached and larvae stages did exist.

The initial goal was to resolve these problems of polyp identification among the three major species of Chesapeake Bay Scyphozoans. In earlier reports, a technique was described whereby the polyps of the three forms could be distinguished on the basis of nematocyst type and dimension.

Fortunately, this technique using nematocysts confirmed the field character, utilizing mouth shape, upon which polyp identification in the earlier phases of the program had been based. In addition, the development of Aurelia and Chrysaora from newly liberated ephyra to medusa was outlined. In this final report, new information facilitating separation of the newly liberated ephyrae is given.

A fourth scyphozoan, Rhopilema verrilli, has been found in lower Chesapeake Bay. This is a poorly known species and only the medusa is described in the literature. We have succeeded in getting this species into culture and descriptions of the planula and polyp stages are being prepared.

A second major objective was to determine the relationship between Chesapeake Bay populations of Chrysaora and Aurelia, and populations in other geographic areas. There has been considerable speculation that geographic races or subspecies may exist, and that important differences in physiology and life history might be expected.

In order to clarify these problems, a series of polyp cultures (detailed elsewhere) has been accumulated from widely separated areas and these are now under study. The results of this effort will be forthcoming in the next contract period.

During the course of the present contract period an extensive bibliography of the Scyphozoa was developed. A draft version was included in a past annual report. The draft version was circulated to other researchers and their assistance sought in finding errors and omissions. The expanded and revised version has been completed and is ready for publication. Because of the length of the bibliography, it is not included in this report. However, copies have been submitted to the National Marine Fisheries Service, in order to obtain clearance for publication.

III. Biochemical and Developmental Studies

The principal focus of this contract has been placed on the polyp stage and the metamorphic processes especially strobilation. Our principal assumption has been that control could ultimately best be effected by breaking one of the metamorphic links.

To these ends we have concentrated a great deal of effort on the biochemistry and metabolism of the polyp stages, especially seeking differences between strobilating and non-strobilating polyps.

During the earliest stages, a great deal of technique development was required. Many of these techniques were worked out using medusae rather than polyps because of the greater amounts of material available. Also this approach allowed us to identify many of the components that would be present in the smaller polyp stages. The techniques relied upon most heavily have been thin-layer and column chromatography with gas-liquid chromatography the principal tool for identification. A good deal of progress in

identification of lipids and amino acids has been reported in earlier annual summaries. More recently acquired information is included elsewhere in this report.

In the final year of the contract the biochemical techniques were refined from a gross to a micro-level so that we are now able to extract and identify components from a single polyp, thus allowing comparisons of a strobilating and non-strobilating polyp. A number of such extractions have been made in recent months; unfortunately, during this same period access to the gas chromatograph was lost. A new gas chromatograph has been obtained for the jellyfish research and is now being installed. As soon as this instrument becomes operational, the back-log of extracts can be analyzed with no further loss of time.

Concurrent with the descriptive biochemistry, a number of experiments directly related to strobilation have been conducted. The distribution of iodide among several major classes of compounds was studies in polyps of Aurelia, by use of the radioactive isotope I^{131}. The presence of iodide has been demonstrated to be necessary for strobilation to take place. Iodide is taken up readily and bound to a number of compounds and thus far the detection of unique iodide compounds formed prior to strobilation is obscured by its general reactivity.

Experiments utilizing synchronously strobilating Aurelia populations have been conducted to measure changes in levels of nucleic acids and proteins. These results are reported elsewhere as are the results of limited studies utilizing inhibitors of RNA, DNA and protein synthesis.

IV. Physiological Studies

The physiological studies undertaken in this contract can be subdivided into three categories:

1. The phenomenon of planula setting and metaporphosis;
2. Measurement of metabolism as reflected by respiration; and
3. The role of amino acids and their relationship to the osmoregulatory mechanisms.

It is obvious that this group of activities is closely related to the biochemical and developmental studies.

The first study conducted under this general activity concerned the temperature and salinity conductions under which the planulae of Aurelia could successfully set and develop into polyps. The data resulting from these studies have already been reported and demonstrated surprisingly broad salinity limits for setting and metamorphosis. Similar studies have also been conducted on Chrysaora.

This report contains preliminary results of setting studies on Cyanea and these experiments included crowding as a variable. Several of these experiments produced contradictory but interesting results and are being repeated.

Respirometric studies utilizing single polyps have proven difficult. Some of the difficulties have resulted from new instrumental techniques which are still being perfected.

The amino acid analyses of the polyp stages of all three species has progressed very satisfactorily. Some of the resultant data have been reported earlier and additional data are included in this report. A manuscript summarizing all of the amino acid

work is in draft preparation and will be submitted shortly for clearance and publication.

V. Jellyfish Mortality Studies

A program directed towards identification of chemical inhibitors capable of blocking metamorphosis or causing mortalities as one of the life stages was initiated at VIMS in July 1970. The program consists of screening a wide variety of chemicals. Many of the substances evaluated to date are enzyme inhibitors known to influence developmental processes in other organisms. These results should interact with the developmental studies in suggesting specific enzyme pathways involved in polyp metabolism and metamorphosis.

From July 1970 through March 1971, approximately 100 compounds were tested. To date, five of those compounds produced significant mortality at 0.1 PPM. All compounds tested and test results are included in the main body of the report. For those compounds that are highly toxic at low concentrations, tests for specificity will be run. Additional screening of compounds will continue.

Suggestions of a disease related mortality were made in an earlier report. We proposed to follow such an event if we observed a similar mortality in the future, recognizing that this would have to be research of opportunity. Such a reoccurrence has not been observed in the final year of the original contract. We intend to watch for this in future summers.

JELLYFISH ECOLOGICAL STUDIES

Field collections under this phase were terminated in June 1970. During the period between April and June 1970, we monitored the set of Cyanea larvae at our shellbag stations, collected special bottom shell samples in search of Aurelia polyps, and gathered quantitative data on seasonal distribution and abundance of medusae. Additional bottom shell samples were collected at several other stations.

Final analysis of all data collected between 1968 and 1970 is underway presently.

1. Setting of jellyfish larvae on oyster shells in wire bags.

Monitoring of jellyfish larval set was continued at all the stations set up between December 1969 and January 1970. Shellbags were replaced and examined at two to three-week intervals at most of the stations. No set had been recorded at any of the stations prior to 1 April 1970.

No polyps were found at any of the stations and no set of larvae was recorded at the Great Wicomico and Ware stations between April and June (Table 1).

Small numbers of Cyanea larval cysts were found at 6 of the 18 stations. Four of these six stations (off Broad Creek, off Thoroughfare, Nandua Creek, and Kings Creek) were located close to the mouth of the tributary involved. The Nandua Creek station was the only one at which larval cysts were found twice in the period. The set recorded at Nandua Creek on 30 April (0.26 cysts per shell per day) was comparatively high. Higher set means had previously

been recorded in 1969 at only three other stations (0.56 at Glouces-ter Point, 0.32 at Dixie and 0.27 at Millenbeck).

The four stations where larval cysts were found in 1970 also showed a set in 1969. The Wilson Creek and Gloucester Point stations, where no set was recorded in 1970 had shown a set in 1969. The two other stations where a set was recorded in 1970 (off Broad Creek and off Thoroughfare) were not sampled in 1969.

At the stations where larval cysts were found in 1970, temperature ranged from 15°C to 23°C in a period covering 21 days (28 April to 19 May). Salinity range was 10.0 to 15.7.

The 1970 data appear to corroborate what was suggested by the 1969 data on the relationship of temperature to set of Cyanea larvae. The larvae set late in April and in May and June when water temperatures are on the rise and generally higher than 15°C. No set of Cyanea larvae has been recorded in July.

II. Abundance of polyps and cysts on the bottom shells.

Bottom shell samples were collected from 37 stations between 1 April and 11 June 1970 (Table 2). Most of the 52 samples collected were part of a special sampling schedule in search of Aurelia polyps but the data represent an addendum to the winter bottom sampling completed on 25 March.

Polyps and podocysts were nearly equal in abundance at most of the stations where the presence of both forms was recorded. This represents a shift from what was recorded during the winter sampling period; during that period of low water temperature (always less then 10°C) the number of podocysts was significantly greater than the number of polyps.

During the first week in April 1970, temperatures at most stations were around 10°C but by the middle of May they had climbed up to around 20°C. The rising temperatures during the period apparently stimulated Chrysaora to emerge from the encysted stage thus accounting for the observed increase in the number of polyps.

As observed in previous sampling periods the number of polyps and podocysts at the West Point station in the Corrotoman River was very high.

Cyanea podocysts were found at 6 stations in the Rappahannock River, 2 in the Ware River and at both stations sampled in the Chesapeake Bay near the mouth of the York River. The largest concentration by far was found at the York Spit light station in Chesapeake Bay where a mean of 41.4 podocysts per shell was recorded on the 20 shells examined. The numbers of Cyanea podocysts found at the other stations were relatively small.

The eight Cyanea podocysts recorded at Morattico Bar in the Rappahannock River on 27 April represent an unusual find. Water salinity was 9.8‰ and the station is 24 miles from the mouth of the river. We have not found Cyanea cysts that far from the bay before now. Salinity at that station was 12.5 early in March, and it is possible that Cyanea larvae could have set there in March although that seems early for such a set.

All eight podocysts at Morattico were found on a single shell. This raises the possibility that that shell might have been transported into the area in the course of oyster farming operations when oysters were moved in from the Great Wicomico River.

The geographic distribution of Cyanea larval cysts at bottom sample stations was similar to that at shellbag stations in that larval cysts were usually found at stations close to the Chesapeake Bay. Seven of the 11 stations where larval cysts were found on bottom shells are located within five miles of the geographical boundary of the tributary with Chesapeake Bay (or Mobjack Bay in the case of the Ware River stations).

The stations in the Chesapeake Bay (Tue Marsh Light and York Spit Light) had a much higher concentration of Cyanea larval cysts than the stations in its tributaries with the exception of Glebe Point on the Great Wicomico. The latter had a mean number of cysts per shell of 81.35; this mean is much higher than any previously recorded by us for a tributary station.

These data provide further support for the conclusion that the Chesapeake Bay proper constitutes the focus of Cyanea polyp populations in the lower Chesapeake Bay estuary. The extent to which the higher salinity waters of its tributaries contribute to the Cyanea population appears to be limited as suggested by most of our data.

III. Identification of polyps in bottom samples by their nematocyst complement

In February and March 1970 we examined subsamples of polyps from many of the bottom shell samples collected during that period using Dr. Calder's technique for the identification of polyps based on nematocyst types. This showed that the large majority of

the polyps collected were Chrysaora; a few polyps were Cyanea and no Aurelia polyps were found.

We repeated this type of examination on subsamples of polyps in bottom samples collected between April and June 1970. These polyps were collected in association with Dr. Calder in an effort to locate Aurelia polyps in nature.

This series of samples was collected in a manner different from our usual procedure for securing bottom shells. Prior to then most of our bottom samples were collected by tonging or dredging and the shells transported to the laboratory in open buckets. No effort was made to save and examine for polyps the water in which the shells were transported. These methods might have caused Aurelia polyps to detach from the shells and be lost from the samples in the water covering the shells in the buckets.

To insure that any Aurelia polyps on the shells collected were not lost the April to June series of samples were collected with the aid of SCUBA diving gear. Shells were picked off the bottom by hand and carefully placed in a small polyethylene bag held in the other hand by the diver. After several shells were in the bag the latter was closed tight by the diver and brought up to the surface where a man on the boat sealed the bag tight and immersed it in a bucket of water.

At the laboratory the shells and water in a bag were emptied into another bucket and both shells and water were carefully examined for polyps. Very few polyps were found loose in the water inside the bags. After the polyps were counted a sub-

sample was removed from the shells and examined and identified using Dr. Calder's technique.

The results from this series of samples paralleled those obtained in the earlier samples (Table 3). Most of the polyps in the samples collected from bay tributaries in this series were Chrysaora; out of 221 polyps examined 214, or 96.8%, were Chrysaora. All polyps examined from the York Spit Light station in the Chesapeake Bay proper were Cyanea. The remainder of the polyps from York Spit Light, which were not examined for nematocyst type, appeared to be Cyanea also because of their similarity in morphology and proximity on the shells to those removed for examination, to each other and to Cyanea podocysts. As in the previous series we found no Aurelia polyps on these samples.

In the February to March 1970 series of bottom samples 90.5% (237 out of 262) of the polyps examined from bay tributary stations were Chrysaora (annual report for period 1 July 1969-31 March 1970). A summary of both series of examinations shows that 462 out of 487, or 94.9%, of the polyps examined from bay tributary stations were Chrysaora and only 5.1% were Cyanea. No Aurelia were found. One-hundred percent of the polyps examined from Chesapeak Bay proper were Cyanea.

Examination of hundreds of polyps from our bottom and shellbag collections in the past two years showed that none of those in the 16-tentacle stage had the circular mouth outline peculiar to Aurelia polyps in the Chesapeake Bay region (as shown by us in the quarterly report for the period 1 October - 31 December 1968).

The results of examinations for mouth shape and examinations for nematocyst complement clearly indicate that nearly all the polyps found in the lower Chesapeake Bay tributaries are Chrysaora polyps. In all our data on polyp distribution and abundance in these tributaries we have avoided reference to the species of the polyps collected because of the possibility that polyps of more than one species might have been included in any specific count.

On the basis of the examinations made to date on mouth shape and nematocyst complement of these polyps, it may be safely stated now that the probability is very high that between 90 and 97 percent of the polyps collected from Chesapeake Bay tributaries are Chrysaora quinquecirrha polyps. Therefore, the data collected may be taken to represent reliable information on the ecology of C. quinquecirrha polyps in the tributaries of lower Chesapeake Bay.

IV. Seasonal distribution and abundance of medusae

A. Counts from VIMS pier at Gloucester Point

We continued to monitor the abundance of medusae at Gloucester Point by making daily counts off one of our piers during weekdays. The counts were made between 11:00 A.M. and 1:00 P.M. E.S.T.

The data are summarized in terms of average number counted for the 5-day period to eliminate the variations that occurred from day to day (Table 4).

Cyanea medusae were present in the area through the middle of May 1970 in fair abundance. Many of the counts recorded were low but this was probably due to the strong winds that prevailed

through April. The wind stirred up the bottom sediments curtailing visibility through the water. This may have also caused medusae to stay in the deeper water away from the pier.

The disappearance of Cyanea medusae after the first week in May 1970 was marked by an apparently sudden mortality in its population. No dead medusae had been noticed up to 8 May. On 11 and 12 May, however, we counted 917 and 350 dead medusae, respectively, without any evidence of live ones. On 13 May we observed just one dead medusae and after that date no more Cyanea medusae, dead or alive, were seen until counts were resumed in 1971.

No medusae of any species were seen for the next three weeks. The first Chrysaora medusae recorded from the pier at Gloucester Point was seen on 9 June.

Chrysaora medusae were very scarce around the VIMS pier in 1971. The highest count on any single day was 10 medusae and the majority of the daily records consisted of counts of 0 to 3 medusae. No Chrysaora medusae were seen after 14 August 1970.

Our field operations were terminated in June 1970, therefore, we have no records of medusae abundance in other areas to compare with the counts made at Gloucester Point. However, in frequent visits made to Wilson Creek and Warehouse Landing in the Ware River it was noted that Chrysaora and Aurelia medusae were present in large numbers in that area. The same may have been true of other areas not visited by us. The scarcity of Chrysaora medusae at Gloucester Point in 1970 cannot be explained.

Aurelia medusae were even scarcer than Chrysaora at the VIMS pier in 1970. Only three were counted during the whole period.

Medusae counts from the pier at Gloucester Point were resumed on 10 February 1971. During the first two weeks the number of Cyanea counted was relatively small. On February 24, however, extremely high numbers of medusae appeared in the waters in front of our campus. A total of 4, 600 was recorded in our counting area but thousands more were evident in the surrounding areas. Since that time large numbers were frequently seen and counted through the end of March.

As in previous years the large majority of the Cyanea medusae seen in 1971 were small. The maximum daily mode estimated was 3.5 inches and most frequently it was estimated to be around 2.5 inches.

B. Counts from moving boat at field stations

Due to termination of our field operations medusae counts at field stations did not extend beyond the middle of June 1970, except for one instance when a count was made at Nansemond Ridge on the James River on 27 July. The counts recorded from the stations visited between April and July 1970 appear on Table 5.

Cyanea medusae continued to be moderatly abundant at Glebe Point on the Great Wicomico River as they had been during February and March. They were still present in the area on 25 May 1970.

Very few Cyanea were seen in any of the other tributaries visited. This was especially true of the James and Rappahannock Rivers. The highest single count at any station was recorded from Kings Creek on the Eastern Shore of Chesapeake Bay on 19 May 1970.

No medusae were seen at Tue March Light in two visits or at York Spit Light on a single visit. Counts made at these stations and at others in Chesapeake Bay proper earlier in the winter of 1969-1970 and in the winter of 1968-1969 resulted in similar observations. More Cyanea medusae are seen near the surface inside the lower Chesapeake Bay tributaries than in the bay proper even though Cyanea polyps are more abundant in the bay than in the tributaries.

The presence of dead Cyanea medusae at several stations visited between the end of April and the first two weeks in May suggests that they were beginning to disappear from the region. It is very likely that all were gone by the end of May. No Cyanea medusae were seen at any of the stations visited in early June.

Chrysaora medusae were observed in the Great Wicomico River on 27 May at two stations: Glebe Point and Haynie Point. These were small, ranging from 0.5 to 4 inches with a mode range of 2 to 2.5 inches. No Chrysaora medusae were observed at any of the other stations in May or June. On July 27 we counted 85 at Nansemond Ridge. These also were small medusae ranging in size from 1 to 3 inches.

The only Aurelia medusae seen during the period were at Nansemond Ridge on 27 July. They ranged in size from 6 to 8 inches.

V. Temperature and Salinity

Temperature and salinity data collected during the period April to July 1970 appear on Table 6.

TABLE 1

Number of Cyanea capillata Polyps and Larval Cysts on Oyster Shells in Suspended Wire Bags, April-June 1970

River and Station	Date Coll.	No. Days Exposed	No. Shells Examined	No. Polyps	No. Larval Cysts
Great Wicomico					
Glebe Point	7 Apr	14	10		
	21 Apr	14	10		
	4 May	13	10		
	25 May	21	10		
Rappahannock					
Totuskey Creek	8 Apr	16	10		
	27 Apr	19	10		
	14 May	17	10		
Bowlers Rock	8 Apr	16	10		
	27 Apr	19	10		
	14 May	17	10		
Morattico	8 Apr	16	10		
	27 Apr	19	10		
	14 May	17	10		
Lancaster Creek	8 Apr	16	10		
	27 Apr	19	10		
	14 May	17	10		
Robinson Creek	8 Apr	22	10		
	28 Apr	20	10		
	12 May	14	10		
Urbanna	8 Apr	22	10		
	28 Apr	20	10		
	12 May	14	10		
Corrotoman River					
West Point	28 Apr	33	10		
	12 May	14	10	0	8
Corrotoman Point	28 Apr	33	10		
	12 May	14	10		
Off Broad Creek	28 Apr	33	10	0	3
	12 May	14	10		
Broad Creek	28 Apr	33	10		
	12 May	14	10		
Piankatank					
Dixie	7 Apr	14	10		
	21 Apr	14	10		
	4 May	13	10	0	1
	25 May	21	10		
Ware					
Wilson Creek	7 Apr	14	10		
	1 May	24	10		
	26 May	25	10		
York					
Pages Rock	1 Apr	16	10		
	5 May	34	10		
	19 May	14	10		
Gloucester Point	17 Apr	17	10		
	1 May	14	10		
	15 May	14	10		
Off Thoroughfare	5 May	35	10	0	10
	19 May	14	10		
Eastern Shore-Ches. Bay					
Nandua Creek	1 Apr	20	10		
	30 Apr	29	10	0	75
	19 May	19	10	0	8
Kings Creek	1 Apr	20	10		
	30 Apr	29	10	0	1
	19 May	19	10		

TABLE 2
Number of Polyps and Cysts on Bottom Shells

April-June 1970

River and Station	Date Coll.	No. Shells	Polyps				Podocysts				Larval Cysts	
			Chrysaora		Cyanea		Chrysaora		Cyanea		Cyanea	
			No.	x̄	No.	x̄	No.	x̄	No.	x̄	No.	x̄
Great Wicomico												
Glebe Point	27 May	20	90	4.50			74	3.70			1627	81.35
Haynies Point	27 May	20	6	0.30			8	0.40				
Fleet Point	27 May	22										
Fleet Point Buoy 6	27 May	29					6	0.21			292	10.07
Whaley Flats	27 May	5										
Rappahannock												
Bowlers Rock	28 Apr	20										
	14 May	20					4	0.20				
Marattico Bar	8 Apr	20	5	0.25			30	1.50				
	27 Apr	20					33	1.65	8	0.40		
	14 May	20										
	2 Jun	20										
Morattico Creek	14 May	20	104	5.20			30	1.50				
Lancaster Creek	14 May	20	133	6.65			62	3.10				
Smoky Point	8 Apr	20										
	2 Jun	20	1	0.05			23	1.15				
Robinson Creek	28 Apr	20	23	1.15			73	3.65	43	2.15	3	0.15
	12 May	30	62	2.07			48	1.60	2	0.67		
Hogg House	8 Apr	20					32	1.60				
Corrotoman River												
West Point	28 Apr	20	34	1.70			269	13.45	10	0.50		
	12 May	20	202	10.10			151	7.55				
	21 May	31	109	3.52			22	0.71			9	0.29
Corrotoman Point	28 Apr	20	8	0.40			10	0.50				
	12 May	20	9	0.45			5	0.25	6	0.30	27	1.35
Carter Creek	21 May	25	68	2.72			74	2.96	3	0.12		
Below Beacon 1	21 May	47[1]							4	0.09	185	3.94
Broad Creek	21 May	5					7	1.40				
Ware												
Above Whse. Ldg.	11 Jun	6							8	1.33		
Warehouse Ldg.	11 Jun	9										
Thos. Green Ground	10 Jun	32							21	0.66	14	0.44
Below Thos. Green Gd.	11 Jun	30	49	1.63	1	0.03	109	3.63			11	0.37
King's Oyster House	10 Jun	20										
Wilson Creek	10 Jun	10										
Mobjack Bay												
Near Can 3	8 Jun	20										
York												
Aberdeen Creek	19 May	20	10	0.50			32	1.60				
Aberdeen Rock	1 Apr	20	5	0.25			78	3.90				
	5 May	19										
	19 May	20										
Pages Rock	1 Apr	20					21	1.05				
	6 May	20	1	0.05			2	0.10			2	0.01
Tillage's Ground	5 Jun	15										
Ellen Island	5 Jun	20										
Off Perrin River	5 Jun	5										
Poquoson												
Below Patricks Creek	19 May	20	5	0.25								
James												
Horsehead Rock	15 Apr	20										
Wreck Shoal	15 Apr	20					9	0.45				
	13 May	20										
White Shoal	16 Apr	20					45	2.25				
Brown Shoal	16 Apr	20					57	2.85				
	13 May	20										
Chesapeake Bay												
Tue Marsh Light	19 May	20									1607	80.35
	5 Jun	32							4	0.13	900	28.13
York Spit Light	8 Jun	20			196	9.80			828	41.40	1451	72.55

[1] = clean, recently planted shells

TABLE 3

Identification fo Polyp[illegible]ased on Nematocyst Types
Bottom Shell Samples, April-June 1970

River and Station	Date Coll.	No. Polyps Counted	No. Polyps Examined	Aurelia	Chrysaora	Cyanea
Great Wicomico						
Glebe Point	27 May	90	20	0	20	0
Haynie Point	27 May	6	6	0	6	0
Rappahannock						
Morattico Bar	8 Apr	5	1	0	1	0
Morattico Creek	14 May	104	15	0	15	0
Lancaster Creek	14 May	133	15	0	15	0
Smoky Point	2 June	1	1	0	1	0
Robinson Creek	28 Apr	23	11	0	10	1
	12 May	62	25	0	25	0
Corrotoman River						
West Point	28 Apr	34	12	0	10	2
	12 May	202	10	0	10	0
	21 May	99	15	0	15	0
Corrotoman Pt.	12 May	9	5	0	15	0
Carter Creek	21 May	68	20	0	20	0
Ware						
Below Thos. Green Gd.	11 June	48	45	0	44	1
York						
Aberdeen Creek	19 May	10	10	0	10	0
Aberdeen Rock	1 Apr	5	5	0	2	3
Poquoson						
Below Patricks Creek	19 May	5	5	0	5	0
Chesapeake Bay						
York Spit Light	8 June	196*	31	0	0	31

*Proximity to each other and to Cyanea podocysts and similarity in morphology indicated that all these polyps were Cyanea.

TABLE 4

Average Daily Number of Medusae Counted on weekly 5-day periods from pier at Gloucester Point, Va.

6 April 1970-2 April 1971

Period	Cyanea			Aurelia			Chrysaora		
	Ave. No. Medusae	Size Range (in.)	Range of Daily Size Modes (in.)	Ave. No. Medusae	Size Range (in.)	Range of Daily Size Modes (in.)	Ave. No. Medusae	Size Range (in.)	Range of Daily Size Modes (in.)
1970									
6 Apr-10 Apr	254	1-6	1-3						
13 Apr-17 Apr	47	1-6	3-4						
20 Apr-24 Apr	56	1-6	2-3.5						
27 Apr-1 May	17	2-4	2-3.5						
4 May-8 May	42	2-4	3						
11 May-15 May	254[1]	1-4	3						
18 May-22 May	0								
25 May-29 May	0								
1 Jun-5 Jun	0								
8 Jun-12 Jun	0						1[2]	5	5
15 Jun-19 Jun							1	4-7	4-5
22 Jun-26 Jun							1[2]	6	6
29 Jun-3 Jul							4	3-7	3-7
6 Jul-10 Jul							2	2-7	3-4
13 Jul-17 Jul							3	2-6	3-4
20 Jul-24 Jul							1	2-6	2-3
27 Jul-31 Jul				1[3]	4	4	1	2-4	2-3
3 Aug-7 Aug							1[2]	3-4	3-4
10 Aug-14 Aug							2[4]	2-3	2-3
17 Aug-21 Aug							0[4]		
24 Aug-28 Aug				1[3]	10	10	0[4]		
31 Aug-4 Sept				0			0		
7 Sept-11 Sept				0			0		
14 Sept-18 Sept				0			0		
21 Sept-25 Sept				0			0		
28 Sept-2 Oct				---			---		
5 Oct-9 Oct				1[1]	3	3			
12 Oct-16 Oct				0			0		
No count made between 16 October 1970 and 10 February 1971.									
1971									
10 Feb-12 Feb	20	1-4	1-1.5						
15 Feb-19 Feb	2	0.5-4	2						
22 Feb-26 Feb	2189	1-5	2-2.5						
1 Mar-5 Mar	63[5]	1-5	2-2.5						
8 Mar-12 Mar	1172[6]	1-5	2-3						
15 Mar-19 Mar	311	1-5	2-2.5						
22 Mar-26 Mar	1765	1-5	2-3						
29 Mar-2 Apr	63	1-5	2-3						

Footnotes:
1= All dead
2= Less than 1 medusae per day
3= Only 1 medusae seen during period
4= Red Tide present
5= Very windy, extremely low tide last 3 days of week
6= Very windy, extremely low tide first 2 days of week

TABLE 5

Medusae Counts at Lower Chesapeake Bay Stations

April-July 1970

(Number observed from moving boat in area 1000' long by 10' wide)

River and Station	Date	No. Medusae Aurelia	Chrysaora	Cyanea
Great Wicomico				
Glebe Point	7 Apr			49
	21 Apr			27
	4 May			7
	25 May			15
	27 May		78	0
Cockrell Creek	27 May			
Haynie Point	27 May		4	
Fleet Point	27 May			1
Rappahannock				
Totuskey Creek	8 Apr			0
	27 Apr			0
	14 May			0
Bowlers Rock	8 Apr			0
	27 Apr			0
	14 May			0
Morattico Bar	8 Apr			0
	27 Apr			0
	14 May			0
	2 Jun			0
Morattico Creek	14 May			0
Lancater Creek	8 Apr			0
	27 Apr			0
	14 May			0
Smoky Point	8 Apr			0
	2 Jun			0
Robinson Creek	8 Apr			0
	28 Apr			39
	12 May			6 (3)*
Urbanna	8 Apr			1
	28 Apr			3
	12 May			0
Hogge House	8 Apr			0
Corrotoman River				
West Point	28 Apr			1
	12 May			4
Corrotoman Point	28 Apr			0
	12 May			1
Off Broad Creek	28 Apr			9 (3)
	12 May			6 (6)
Broad Creek	28 Apr			8
	21 May			0
Piankatank				
Dixie	7 Apr			2
	21 Apr			7
	4 May			0
	25 May			0
Ware				
Wilson Creek	7 Apr			0
	1 May			0
	26 May			0
Mobjack Bay				
Near Can 3	8 Jun			0
York				
Aberdeen Rock	5 May			2
Pages Rock	1 Apr			12
	5 May			4
	6 May			4
	11 May			0
	19 May			0
Tillage's Ground	5 Jun			0
Gloucester Point	17 Apr			22
	1 May			2 (2)
	11 May			0
	15 May			0
Ellen Island	5 Jun			0
Off Perrin River	5 Jun			0
Off Thoroughfare	5 May			27
	11 May			0
	19 May			0
Poquoson				
Below Patricks Creek	19 May			0
Chisman Creek	19 May			0
James				
Horsehead Rock	15 Apr			0
	8 Jun			0
Wreck Shoal	15 Apr			0
	13 May			0
	9 Jun			0
White Shoal	16 Apr			0
Brown Shoal	16 Apr			4
	13 May			0
	9 Jun			0
Nansemond Ridge	27 Jul	85	7	0
Eastern Shore-Ches. Bay				
Nandua Creek	1 Apr			1
	30 Apr			3 (2)
	19 May			153
Kings Creek	1 Apr			7
	30 Apr			14
	19 May			0
Chesapeake Bay				
Tue Marsh Light	19 May			0
	5 Jun			0
York Spit Light	8 Jun			0

* No. in parentheses indicates no. dead out of total count

TABLE 6
Temperature and Salinity at Shellbag and Bottom sample Stations
April-July 1970

River and Station	Date	Temperature C	Salinity o/oo
Great Wicomico			
Glebe Point	7 Apr	10.0	12.93
	21 Apr	10.6	13.05
	4 May	17.5	11.37
	25 May	25.2	11.33
	27 May	24.9	11.35
Cockrell Creek	27 May	20.8	13.51
Haynie Point	27 May	23.1	12.05
Fleet Point	27 May	20.5	13.19
Fleet Point Buoy 6	27 May	21.0	12.68
Rappahannock			
Totuskey Creek	8 Apr	10.8	3.24
	27 Apr	11.4	3.06
	14 May	24.5	4.13
Bowlers Rock	8 Apr	10.8	4.35
	27 Apr	10.9	4.37
	14 May	25.1	5.26
Morattico Bar	8 Apr	12.1	9.81
	27 Apr	10.4	9.56
	14 May	25.0	8.28
	2 Jun	23.8	9.41
Morattico Creek	14 May	23.6	8.54
Lancaster Creek	27 Apr	11.3	8.12
	14 May	25.8	7.69
Smoky Point	8 Apr	11.8	11.68
	2 Jun	23.5	11.28
Robinson Creek	8 Apr	10.2	11.96
	28 Apr	17.1	11.42
	12 May	22.2	10.91
Urbanna	8 Apr	11.2	11.20
	28 Apr	17.1	11.42
	12 May	22.0	9.59
Hogg House	8 Apr	11.1	14.35
Corrotoman River			
West Point	28 Apr	18.3	12.50
	12 May	23.1	11.88
	21 May	24.0	11.85
Corrotoman Point	28 Apr	17.4	12.76
	12 May	21.0	12.08
Carter Creek	21 May	20.5	12.06
Below Beacon 1	21 May	20.5	12.20
Off Broad Creek	28 Apr	15.0	12.77
	12 May	21.2	12.16
Broad Creek	28 Apr	16.5	12.81
	12 May	----	12.31
	21 May	22.8	11.95
Piankatank			
Dixie	7 Apr	10.0	11.00
	21 Apr	12.2	12.72
	4 May	15.5	10.04
	25 May	23.9	11.53

River and Station	Date	Temperature C	Salinity o/oo
Ware			
Warehouse Ldg.	11 Jun	26.5	12.75
Thos. Green Ground	10 Jun	25.1	14.61
Below Thos Green Gd.	11 Jun	27.7	14.61
King's Oyster House	10 Jun	26.4	15.47
Wilson Creek	7 Apr	10.2	15.20
	1 May	22.8	13.20
	26 May	25.8	15.48
	10 Jun	26.0	15.92
Mobjack Bay			
Near Can 3	8 Jun	----	18.55
York			
Aberdeen Creek	19 May	21.4	11.82
Aberdeen Rock	1 Apr	9.2	13.01
	5 May	18.0	10.75
	19 May	19.1	14.70
Pages Rock	1 Apr	9.2	14.01
	5 May	17.2	12.41
	19 May	20.5	13.24
Tillage's Ground	5 Jun	24.0	17.16
Gloucester Point	17 Apr	12.6	13.98
	1 May	17.7	14.69
	15 May	20.0	15.37
Ellen Island	5 Jun	23.8	17.31
Off Perrin River	5 Jun	23.0	18.49
Off Thoroughfare	5 May	16.0	14.84
	19 May	19.3	15.16
Poquoson			
Below Patricks Creek	19 May	20.2	16.36
James			
Horsehead Rock	15 Apr	12.2	3.95
	8 Jun	24.8	5.84
Wreck Shoal	15 Apr	12.0	5.86
	13 May	25.5	-----
	9 Jun	24.8	9.53
White Shoal	16 Apr	12.8	9.76
Brown Shoal	16 Apr	11.8	13.28
	13 May	25.0	-----
	9 Jun	24.0	15.46
Nansemond Ridge	27 Jul	27.2	-----
Eastern Shore --- Chea Bay			
Nandua Creek	1 Apr	9.9	15.81
	30 Apr	20.8	15.61
	19 May	20.4	15.70
Kings Creek	1 Apr	10.0	18.73
	30 Apr	19.4	16.55
	19 May	21.2	18.75
Chesapeake Bay			
Tue Marsh Light	19 May	19.8	15.09
	5 Jun	24.8	17.61
York Spit Light	8 Jun	23.5	19.86

TAXONOMIC STUDIES

The principal goals of this phase to date have been to resolve problems in the identification of Chesapeake Bay scyphozoans. In earlier reports, a technique was described whereby the polyp or scyphistoma stage of the three common species may be distinguished, and the development of Aurelia and Chrysaora from newly liberated ephyra to medusa stages was outlined. In this report, new information facilitating separation of the newly-liberated ephyrae of Aurelia and Chrysaora is given. The nematocysts of the four species of medusae occurring in the bay are compared, and their potential use in the identification of questionable material is discussed. The complete development of Chrysaora from ephyra to medusa is outlined in greater detail than previously. Preliminary observations on the planula and polyp stages of Rhopilema are given. A polyp collection has been accumulated for planned studies comparing various geographic populations of the relevant species. A major part of the effort expended in this phase of the program during the past year has been directed toward expanding and revising a bibliography on the Scyphozoa, and preparing it for publication. This task has now been completed.

Nematocysts of newly liberated ephyrae

Aurelia aurita and Chrysaora quinquecirrha are summer species, occurring together in some areas of the Chesapeake Bay system. In the previous report the development of both species from ephyra to medusa stages was described so that the two could be distinguished at any developmental stage. While the two species become progressively easier to separate as they develop, the

young ephyrae are less differentiated and more easily confused. The purpose of this study was to determine the utility of the nematocysts as an aid in the identification of the very young ephyrae.

Nematocysts were examined in newly liberated ephyrae of A. aurita and C. quinquecirrha, obtained from strobilae isolated from laboratory cultures. Distinct differences are present in the cnidome of the ephyrae (Table 1) that immediately distinguish the two species of this stage. Chrysaora ephyrae possess four types of nematocysts ("a" atrichs, "A" atrichs, holotrichs and euryteles) while Aurelia ephyrae have only two types ("a" atrichs and euryteles). Holotrichs, lacking in Aurelia, were present in 16 conspicuous batteries, one at the base of each lappet, in Chrysaora. The "A" atrichs, also present in Chrysaora but absent in Aurelia, are of less taxonomic value because they are smaller and fewer in number than the holotrichs, and are therefore less conspicuous. The "A" atrichs of Chrysaora were found mainly in the region of the manubrium, although they were occasionally observed elsewhere.

Nematocysts were also examined in ephyrae of Chrysaora liberated in the laboratory from strobilae collected in the Corrotoman River (Table 2). These did not differ in their cnidome from specimens obtained in laboratory cultures. Similar comparisons were not possible for Aurelia because strobilae of this organism have not yet been found in nature from this area.

Nematocysts of the medusae

The four species of jellyfish indigenous to Chesapeake Bay are usually easy to distinguish unless they are in extremely poor condition. Occasionally, identification of incomplete specimens is needed. Specific identification of such material, or even

determination of whether the remains are those of a scyphozoan, is often difficult or impossible based on gross morphology alone. The season of occurrence could be a key to identification, but there are overlaps in the seasonality of most, if not all, of the species. The purpose of this study was to determine the differences in the nematocyst of the jellyfish of the bay, and their utility in separating the four species. It should also be possible, using the nematocysts, to determine from analysis of gut contents whether a suspected predator has actually ingested a scyphozoan (polyp or medusa), and to identify the ingested species if it is not known.

Aurelia - According to Weill (1934), the cnidome of Aurelia aurita consists of citrichous isorhizas ("a" atrichs) and microbasic heterotrichous euryteles. The same cnidome was observed in Chesapeake Bay specimens from the York River, Virginia (Table 3), although the isochizas varied in morphology from the typical apple-seed shaped "a" atrichs to the elongate " " atrichs. Of the four species of scyphomedusae in the bay, only Aurelia lacks holotrichs.

Chrysaora - Sutton and Burnett (1969) reported on the nematocysts of Chesapeake Bay Chrysaora, employing both light and electron microscopy. Their study was directed more towards an anatomical understanding of the nematocysts than towards taxonomy.

Following Weill's (1934) classification, three categories of nematocysts were identified in Chrysaora here during July 1970 (Table 4). These were atrichous isorhizas, holotrichous haplonemes and microbasic heterotrichous euryteles. The isorhizas were of two types, "a" atrichs and "A" atrichs. All nematocyst types were well represented in the species. The size of the nematocysts is significant taxonomically, particularly the holotrichs and "A" atrichs,

which are much larger than those in the other species in which they occur.

Cyanea - The nematocysts of Cyanea capillata have been described previously by Weill (1934) and Papenfuss (1936) for the northern form Cyanea capillata arctica. Data are presented here (Table 5) for Chesapeake Bay specimens of C.c. fulva collected in the York River. Three categories of nematocysts were identified, atrichons isorhizas, holotrichous haplonemes and microbasic heterotrichous euryteles. Three subcategories of isorhizas were observed, "a" atrichs, "A" atrichs, and " " atrichs. In these medusae the "a" atrichs and " " atrichs appeared to intergrade in morphology. Holotrichs were relatively rare in the examined material.

Rhopilema - The nematocysts of this species have not been described before. Rhopilema verrilli is a rare and poorly known medusa rang-from southern New England to North Carolina and the northern Gulf of Mexico. Several specimens were collected in Virginia waters during autumn and winter of 1970-71. Three categories of nematocysts were found, atrichous isorhizas, holotrichous haplonemes and microbasic heterotrichous euryteles (Table 6) of the various types of isorhizas, only "a" atrichs were present. Despite a size range exceeding an order of magnitude in the six medusae used in nematocyst studies (from 3 to 45 cm in diameter), the nematocysts of a given category were relatively uniform in size from specimen to specimen (Table 7). The most conspicuous nematocysts were the holotrichs, which were particularly abundant on the appendages. Holotrichs were very small in this species and can be immediately distinguished from those in Cyanea and Chrysaora by their size.

The toxin in nematocysts of Rhopilema is evidently mild. No sting was detectable following contact with any of the living medusae examined in this study.

In summary, there are differences in the types (Table 8) and sizes of the nematocysts in the four species of jellyfish in Chesapeake Bay. Examination of the nematocysts, as long as these are present, should provide clues to the identity of questionable material.

Development of Chrysaora

The early development of Chrysaora was summarized in the previous report. Further studies have been made since then on sea nettle development, the results of which are described here.

Observations on laboratory-reared medusae have been correlated with the development of specimens from nature. Growth was followed through six stages, successive stages being characterized by the appearance of or change in a morphological character. The medusae pass through stages having one, three and five tentacles per octant, and may attain a stage having seven or even nine tentacles per octant. The number of lappets increases following an increase in the number of tentacles.

Stage I (Fig. 1) - Newly liberated ephyra about 2.0-3.5 mm wide from lappet-tip to lappet-tip, margin typically with eight pairs of lappets and eight rhopalia, lappets slender, pointed distally, terminating in a cap of nematocysts. Ocular clefts deeply cut, reaching well over half-way along the ephyral arms, U- or V-shaped, wider near the lappet-tips than at the base. Manubrium short, quadrate, gastrovascular cavity with 16 broad radial pouches separated by gastric septa, precursors of the gastric cirri present interradially, primary tentacles absent or their precursors present as faint projections at the base of each tentacular cleft. Mesoglea thin, exumbrella with scattered nematocyst batteries, including a prominent battery of holotrichous isorhizas at the base of each lappet.

Remarks: Newly-liberated ephyrae from laboratory-reared strobilae were indistinguishable from those liberated from strobilae collected at West Point on the Corrotoman River, Virginia. The ephyrae were larger than those described by Littleford (1939) and much smaller than indicated in Cones' (1969) figures. However, specimens examined here corresponded in size with measurements of ephyrae given by Cargo and Schultz (1966).

Young ephyrae of C. quinquecirrha are similar to Kakinuma's (1967) description of Dactylometra pacifica (=Chrysaora melanaster) from Japan at a comparable stage.

Stage II (Fig. 2) - Ephyra with primary tentacles present, one oral arm developing at each perradial corner of the large manubrium. In advanced specimens, primary tentacles prominent, gastric cirri present interradially, one or more per quadrant, tentacular pouches of the gastrovascular cavity perceptably broader distally than the rhopalar pouches. Lappets remaining pointed distally but distinctly broader, their lateral edges becoming folded downward. Oral arms more or less distinct, each capable of independent movement.

Remarks: This stage is characterized by the appearance of the primary tentacles. Under laboratory conditions these tentacles were apparent about three days after liberation. During this stage the gastric cirri appear, the primary tentacles develop considerably, and the lappets increase significantly in breadth.

Stage III (Fig. 3) - Lappets spade-shaped, distinctly concave on the subumbrellar surface and curved downward toward the subumbrella with the adrhopalar edges occasionally overlapping. Original lappet-tips, when evident, asymmetrically placed, being located nearer the ocular cleft than the tentacular cleft. Each lapper with a more or less distinct groove on the exumbrellar surface. Radial muscles of the exumbrella conspicuous, including four perradial, four interradial, and eight adradial muscles, adradial muscles bifurcating distally. Several well-developed gastric cirri per quadrant, manubrium relatively large, oral arms distinct, becoming crenulated in advanced specimens.

Exumbrella with a depression developing adjacent to each rhopalium.

Remarks: With the folding under of the lappets, specimens lose the appearance of the ephyra. Under laboratory conditions this stage was reached within two weeks.

Stage IV (Fig. 4) - Secondary tentacles present between the primary tentacles and rhopalia, one arising from each corner of the tentacular pouches of the gastrovascular cavity. Notches appearing at the tip and near the base of the lappets adjacent to the secondary tentacles, each of the original 16 lappets thus becoming divided into two to form 32 lingueform lappets. Periphery of the exumbrella with 32 radial grooves, one pair, lateral to each of the eight rhopalia, extending outward along the ocular lappets, and a second pair lateral to each of eight primary tentacles, extending outward to the tentacular lappets, nematocyst batteries few or absent in these grooves. Exumbrellar depression adjacent to each rhopalium deeper, with few or no nematocyst batteries. Oral arms long and frilly.

In advanced specimens, four subgenital pits present interradially, gonads becoming developed. Secondary tentacles well-developed. Gastric septa usually marked by 16 white lines on the subumbrella.

Remarks: This stage is marked by the appearance of the secondary tentacles, attained in laboratory-reared specimens about 25 days after liberation. Medusae usually become sexually mature during this stage.

Stage V (Fig. 5) - Tertiary tentacles present lateral to the secondary tentacles, lappets adjacent to the tertiary tentacles becoming notched, then divided into two, the margin having a total of 48 lappets.

Remarks: With the development of the 16 tertiary tentacles and the clefting of the lappets adjacent to these tentacles, the medusa reaches the "Dactylometra" stage, having 40 tentacles and 48 lappets.

Stage VI (Fig. 6) - Medusa with more than five tentacles and 6 lappets per octant.

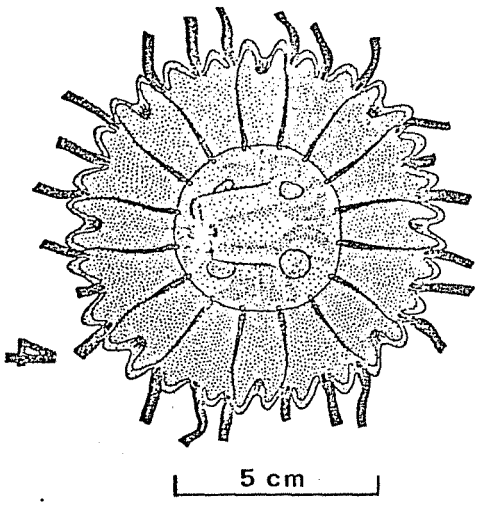
Remarks: The presence of tentacles in addition to the primaries, secondaries and tertiaries mark this stage. Stage VI medusae, having as many as nine tentacles and ten lappets per octant, were common in the Broadkill River, Delaware, on 22 August 1970.

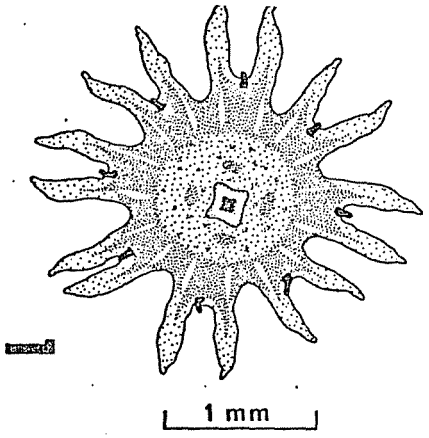
FIGURES 1-6

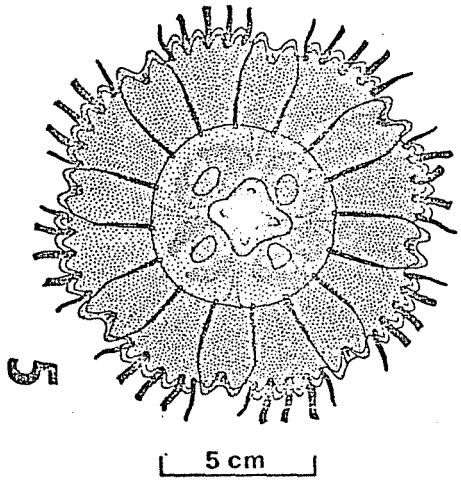
Development of *Chrysaora*

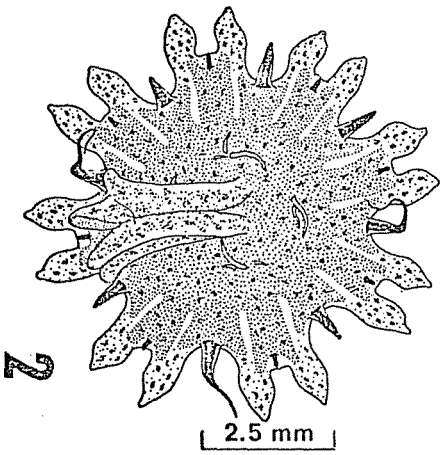
1. Stage I, lab-cultures.
2. Stage II, lab-reared.
3. Stage III, lab-reared.
4. Stage IV, Great Wicomico River.
5. Stage V, Hillsborough Bay, Fla.
6. Stage VI, Broadkill River, Del.

Stages 4-5 shown with lappets extended, tentacles cut off near their origin, and oral arms removed.


4
5 cm


1
1 mm


5
5 cm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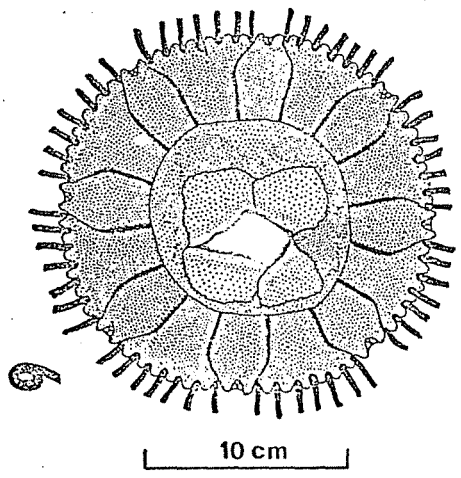
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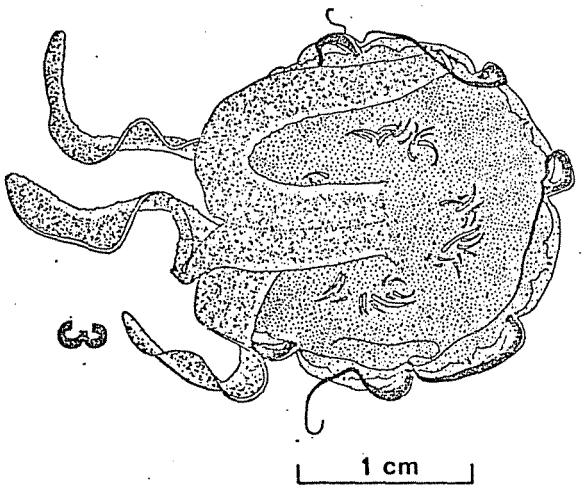

3
1 cm

Life history of Rhopilema

Only the medusa stage of this species has been described in the literature. Planulae were obtained 24 December 1970 from a medusa collected on the eastern shore of Virginia. Some preliminary observations are given here on the planula, and on the polyps reared from the planulae. Studies made on the various stages in the life history of Rhopilema are being continued to determine ways of the other three species.

Planula - The planulae develop within tissue of the gonad, rather than being carried on the oral arms as in Aurelia and Cyanea. They are elongate with blunt ends (Fig. 7), the anterior end being broader than the posterior. They measure about 165-310 u long, 75-100 u die, are whiteish in color, and swim actively by means of cilia. T wo types of nematocysts are present, "a" atrichs and euryteles, with the former being the more commone of the two. Measurements are as follows:

"a" atrichs.....5.0-6.9 X 3.5 -4.1 u (undischarged)

euryteles.....7.3-8.8 X 4.8-5.7 u (undischarged)

Most of the planulae set and metamorphosed into tiny polyps within 7-10 days after being artificially dislodged from the gonad of the medusa. Cultures were maintained at 12C in fingerbowls containing filtered York River seawater of about 20‰. Brine shrimp fed once or twice weekly, were used as food.

Polyp - After setting, polyps were held at 12 C for three weeks, then were maintained at 10 C for 10 weeks. At this temperature, the polyps grew slowly. After being transferred from 10C to room temperature, the polyps grew more rapidly and appeared healthier.

Young polyps are goblet-shaped, with a very slender stalk that is sheathed in a thin cuticle. The proboscis is dome-shaped and very prominent (Fig. 8). The tentacles are filiform and occur in one whorl. At the four-tentacle stage, polyps reach about 500 u long from aboral disc to mouth. The nematocysts in polyps at this stage are similar to those of the planula:

"a" atrichs.....5.0-6.7 X 3.7-4.0 u (undischarged)

euryteles.....7.0-8.1 X 4.9-5.7 u (undischarged)

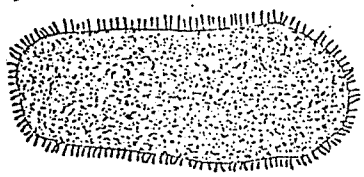
Older polyps are goblet - a sac-shaped, with up to 16 filiform tentacles in a single whorl. The proboscis is very long (Fig. 9) and nearly cylindrical when extended. The nematocysts of fully-formed polyps have not yet been studied in detail.

Polyps of this species form podocysts (Fig. 10) measuring about 280-350 u in diameter. The podocysts are golden in color when newly formed.

FIGURES 7-10

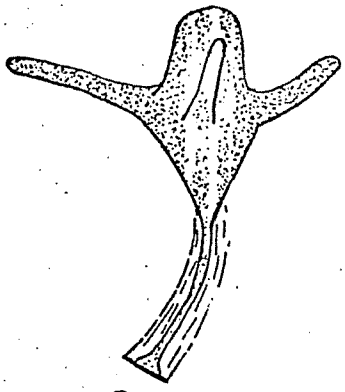
Non-medusoid stages of *Rhopilema verrilli*

7. Planula.
8. Young polyp, 4-tentacle stage.
9. Older polyp.
10. Podocysts, top and lateral views.



7

300 μ



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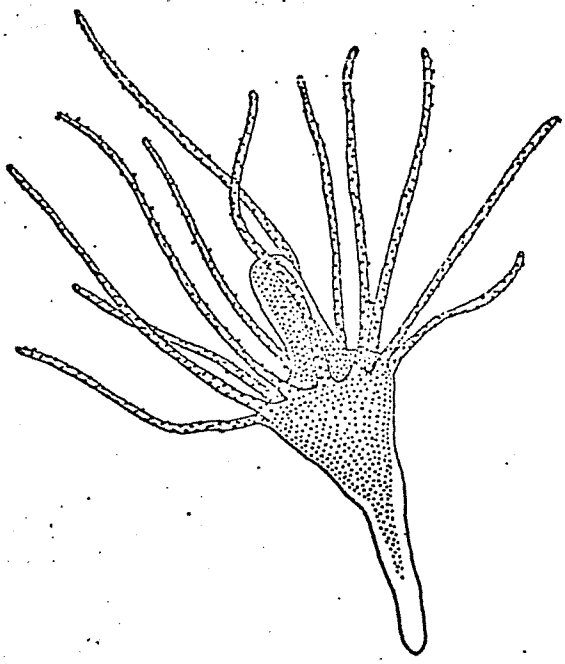
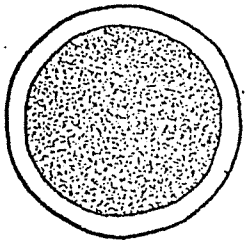


9

1000 μ



10

300 μ

Polyp Collections

Emphasis in the taxonomic study of Chesapeake Bay scyphozoans is being shifted from research on identification problems to defining the similarities and differences between populations of the same species from various geographic areas. The objective of such studies is to determine whether observations on a given species from other regions actually apply to the same species in Chesapeake Bay. Polyp collections for this work are being accumulated. The collections at present consist of the following:

Chrysaora	Delaware	from "red" phase
	Delaware	from "white" phase
	Virginia	from "white" phase
	North Carolina	from "brown" phase
Aurelia	England	
	Massachusetts	
	Delaware	
	Virginia	
	Texas	
Cyanea	Virginia	
Rhopilema	Virginia	

Bibliography on the Scyphozoa

Of the work undertaken under the taxonomic phase of the VIMS Jellyfish Program during the past fiscal year, preparation of a revised and expanded bibliography has received the greatest expediture of time. This task is now essentially completed, and the bibliography is ready for printing. Only a minor effort should be needed to keep the bibliography up to date.

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TABLE 1. Nematocysts of the newly liberated ephyrae of *Aurelia aurita* and *Chrysaora quinquecirrha*, from laboratory cultures.

	Aurelia	*Chrysaora*
"a" atrichs		
No examined	25	25
length (u)	4.0-5.9	4.0-6.9
width (u)	3.0-4.0	3.0-5.0
mean length (u)	5.5	5.9
mean width (u)	3.7	3.9
greatest L:W ratio	2.00:1	2.00:1
smallest L:W ratio	1.25:1	1.25:1
mean L:W ratio	1.49:1	1.52:1
"A" atrichs		
No. examined	0	25
length (u)		7.9-10.9
width (u)		5.0-6.9
mean length (u)		9.1
mean width (u)		6.2
greatest L:W ratio		1.80:1
smallest L:W ratio		1.14:1
mean L:W ratio		1.48:1

TABLE 1 (Cont'd)

	Aurelia	Chrysaora
Holotrichs		
No. examined	0	25
length (u)		9.9-17.8
width (u)		8.9-14.9
mean length (u)		11.6
mean width (u)		11.1
greatest L:W ratio		1.30:1
smallest L:W ratio		1.00:1
mean L:W ratio		1.11:1
Euryteles		
No. examined	25	25
length (u)	6.9-11.9	8.9-12.9
width (u)	5.0-8.9	6.9-9.9
mean length (u)	9.7	10.6
mean width (u)	7.9	8.5
greatest L:W ratio	1.43:1	1.43:1
smallest L:W ratio	1.11:1	1.11:1
mean L:W ratio	1.22:1	1.26:1

TABLE 2. Nematocysts of the newly liberated ephyrae of Chrysaora quinquecirrha, obtained from strobilae collected in nature.

	"a" atrichs	"A" atrichs	Holotrichs	Euryteles
No. examined	25	25	25	25
length	5.4-7.4	8.2-11.9	9.6-14.1	8.9-10.9
width	3.0-4.7	5.0-7.7	8.0-12.1	6.8-8.3
mean length	6.2	9.6	11.8	10.2
mean width	4.1	6.0	10.2	7.8
greatest L:W ratio	2.33:1	1.93:1	1.30:1	1.38:1
smallest L:W ratio	1.38:1	1.42:1	1.04:1	1.20:1
mean L:W ratio	1.58:1	1.59:1	1.16:1	1.30:1

Table 3. Data on nematocysts from medusae of Aurelia aurita.

	"a" atrichs	" " atrichs	euryteles
No. examined	25	25	25
length (u)	5.6-6.9	6.8-9.0	10.0-11.8
width (u)	3.8-4.7	2.7-3.5	6.0-6.9
mean length (u)	6.3	8.0	10.8
mean width (u)	4.1	3.1	6.5
greatest L:W ratio	1.82:1	3.00:1	1.78:1
smallest L:W ratio	1.40:1	2.26:1	1.54:1
mean L:W ratio	1.56:1	2.65:1	1.65:1

Table 4. Data on nematocysts from medusae of Chrysaora quinquecirrha.

	"a" atrichs	"A" atrichs	Holotrichs	Euryteles
No. examined	25	25	25	25
length	7.6-9.9	19.4-28.9	15.8-25.3	12.7-16.5
width	4.0-5.0	13.3-18.8	13.9-22.8	7.7-9.7
mean length	8.9	25.2	20.4	14.6
mean width	4.5	16.9	18.5	8.6
greatest L:W ratio	2.25:1	1.76:1	1.15:1	1.85:1
smallest L:W ratio	1.74:1	1.37:1	1.06:1	1.57:1
mean L:W ratio	1.99:1	1.48:1	1.10:1	1.69:1

TABLE 1. Molar percentage of FAA in *Aurelia aurita* polyps.

	10	15	20	25	30	35
Taurine	4.2	7.8	11.3	14.2	10.4	9.7
Aspartic acid	1.1	0.6	0.7	0.4	0.7	0.6
Threonine	1.1	0.7	0.7	0.7	0.9	1.0
Serine	1.1	1.5	1.6	1.4	1.8	2.4
Glutamic acid	20.5	8.5	7.6	6.1	5.2	5.3
Proline	-	0.4	0.8	1.0	2.1	4.2
Glycine	9.4	31.6	44 2	46.2	41.1	33.0
Alanine	2.1	1.4	1.8	1.2	1.5	2.2
Valine	0.6	1.1	1.3	1.5	1.9	2.2
Cystine	-	-	-	-	-	-
Methionine	0.6	0.4	0.3	0.6	0.4	0.4
Isoleucine	0.4	0.8	0.8	0.9	1.1	1.1
Leucine	0.5	0.8	0.9	0.9	1.0	0.8
Tyrosine	-	0.6	0.6	0.7	0.8	0.9
Phenylalanine	-	0.6	0.4	0.5	-	-
Beta alanine	-	1.3	6.7	7.7	16.4	21.7
Ornithine	-	0.6	0.4	0.3	0.3	0.4
Lysine	14.4	4.1	2.0	1.2	1.1	0.9
Tryptophan	-	-	0.3	0.3	0.3	0.4
Histidine	0.9	0.4	0.3	0.3	0.3	0.5
Arginine	2.0	1.1	1.1	1.2	1.6	1.4
Unidentified	41.2	35.7	16.2	12.9	11.0	10.8
Total NM per 65 polyps	442	1394	1965	2932	2775	2267

TABLE 2. Molar percentage of free amino acids (FAA) in laboratory grown Chrysaora quinquecirrha polyps

Salinity ‰	10	15	20	25	30
Cysteic acid	3.2	3.4	2.1	0.9	1.1
Taurine	1.3	1.3	1.7	1.0	1.9
Aspartic acid	1.1	1.1	0.8	0.6	0.5
Threonine	0.6	0.5	0.7	0.6	0.8
Serine	1.2	1.3	2.5	1.6	2.6
Glutamic acid	7.5	8.1	5.1	3.3	3.5
Proline	-	-	-	0.5	0.6
Glycine	25.3	69.2	76.8	83.9	82.2
Alanine	0.8	1.0	1.1	1.0	1.1
Valine	0.4	0.2	0.6	0.4	0.7
Cystine	0.8	0.8	0.3	0.8	0.2
Methionine	0.3	-	-	-	-
Isoleucine	0.2	-	0.5	0.1	0.2
Leucine	0.4	0.1	0.3	0.6	0.4
Tyrosine	0.2	0.3	0.6	0.3	0.5
Phenylalanine	0.4	0.3	0.4	0.6	0.1
Ornithine	0.3	0.2	1.0	0.4	0.2
Lysine	3.6	0.2	1.0	0.4	0.2
Tryptophan	-	-	-	-	
Histidine	1.0	0.8	0.6	0.6	0.2
Arginine	-	-	-	-	0.9
Unidentified	51.5	9.5	4.0	2.9	2.7
NM/Mg Protein	758	774	1390	1860	2080

TABLE 4. Molar percentage of free amino acids from polyps of Aurelia from Woods Hole, Mass and Cyanea capillata from Virginia.

	Aurelia	*Cyanea*
Salinity ‰	21.7	20
Cysteic acid	2.1	7.9
Taurine	3.0	4.7
Aspartic acid	0.3	2.9
Threonine	0.7	1.7
Serine	2.6	2.5
Glutamic acid	5.5	13.7
Proline	1.3	6.3
Glycine	68.2	11.8
Alanine	1.8	2.6
Valine	0.7	2.8
Methionine	0.3	0.6
Isoleucine	0.4	1.8
Leucine	0.9	3.0
Tyrosine	1.5	0.1
Phenylalanine	0.3	1.5
Ornithine	0.9	0.5
Lysine	1.0	2.3
Histidine	0.3	0.9
Arginine	0.5	6.2
Unidentified	7.8	26.2
MM/Mg Protein	1530	780

JELLYFISH MORTALITY STUDIES

I. INTRODUCTION

A program directed toward identification of chemical inhibitors capable of causing mortality of jellyfish, at one of its life stages, was initiated at VIMS in July 1970. The studies are designed to find the concentrations of various substances which may cause death of the polyp, planula or medusa stage. Sublethal effects are also monitored. The purpose of our study is to find substances that will inhibit the metabolism of jellyfish cells to the extent of causing death, with negligible damage to other organisms in the ecosystem. The specificity exhibited by enzyme systems of organisms provides the opportunity of achieving such selective poisoning.

The program consists of screening a wide variety of chemicals. Many of the substances evaluated to date are enzyme inhibitors known to influence developmental processes in other organisms. Additional compounds are being screened to establish guidelines on the effects of major groups of chemicals. Substances to be evaluated in the future will be selected by a process of convergence toward a particular chemical structure or active component on the basis of results obtained in these preliminary tests.

II. MATERIALS AND METHODS

A. Experimental animals

Tests conducted to date have been on laboratory-reared polyps of Chrysaora quinquecirrha and Aurelia aurita. Two tests

have been conducted on C. quinquecirrha medusae and one on it's ephyrae. Several tests have been conducted on A. aurita ephyrae.

Polyps used were obtained from mature medusae of both species collected between August and September 1970 at Wilson Creek in the Ware River, Virginia.

Polyps of A. aurita were obtained by stripping planula from the oral arms of the medusae in the field. The planulae were brought to the laboratory in a plastic bucket. In the laboratory the bucket contents were diluted with river water and emptied into several glass fingerbowls. Dilution was necessary to avoid overcrowding the polyps in the fingerbowls. The larvae set on the bowl surfaces and the resulting polyps were kept there until used in tests. Once a week water was changed in the bowls and polyps fed brine shrimp.

Polyps of C. quinquecirrha were obtained from sexually mature male and female medusae. Five or six medusae of each sex were placed together in a bucket of river water. Medusae were kept in the buckets overnight on a large, specially built, shaker table which maintained the bucket contents under continuous gentle agitation.

After twelve hours on the shaker table the content of the bucket was concentrated by straining through a No. 20 plankton net. The concentrate containing embryos and planulae were emptied into glass fingerbowls holding river water and small glass slides (approximately 1.5 X 1.5 cm.) on the bottom. Planulae set on the slides and there developed into polyps.

Each slide with attached polyps was transferred from the fingerbowls into the individual compartment (4.0 X 4.3 X 5.2 cm.) of a clear plastic box where they were held until used in tests. Once a week water in each compartment was changed and polyps fed brine shrimp.

The ephyrae used in tests with that life-stage were collected from the above fingerbowls and plastic boxes whenever the polyps held in them strobilated. Only Aurelia polyps strobilated among the laboratory-reared polyps. A large number of ephyrae were released during the last days of December 1970 and the first two weeks in January 1971. As many tests as possible were conducted immediately after the ephyrae were released.

The single test with Chrysaora ephyrae was conducted with ephyrae liberated in the laboratory in August 1970 by strobilating polyps brought in from the field.

C. quinquecirrha medusae used in toxicity tests were from Wilson Creek. Wilson Creek medusae were used for tests and for raising polyps because they were very abundant there and were easily accessible via the Joseph residence's pier. Medusae were very scarce in the York River in 1970.

B. Chemicals

Chemicals used to date were acquired from five different sources. Two groups were received from federal government agencies and three groups from commercial outlets.

A group of chemicals (primarily nitrophenols and nitrosalicylaniles) which displayed relatively high toxicity to larval sea lampreys in the Great Lakes region were supplied by Dr. E.

Louis King, Jr. of the Hammond Bay Biological Station, Michigan (Bureau of Sport Fisheries and Wildlife). A group of pesticide standards was obtained from the Pesticides Repository of the U. S. Public Health Service at Perrine, Florida.

C. Experimental procedure

All tests were to be conducted in filtered York River water at a temperature of 24° C and a salinity of 17‰. Slight variations in room temperature occurred within a 24-hour period and it fluctuated between 23° and 25° C. Salinity also varied from test to test ranging between 16.8 and 17.3‰.

All animals used were acclimated to experimental temperature and salinity for 24 to 48 hours prior to start of a test.

A concentrated stock solution of the chemical to be tested was prepared in distilled water or an appropriate carrier (only acetone and alcohol have been used so far). The stock solution was diluted to 100 PPM in river water and all test concentrations prepared from the latter.

Most tests were conducted at concentrations of 10, 5, and 1 PPM. Chemicals which showed lethal effects at 1 ppm were reevaluated at 0.5 and 0.1 ppm. If results from a test were questionable the test was repeated.

Control animals were maintaining during all studies in untreated filtered river water. Control experiments were also conducted in one or more concentrations of the carrier used. These concentrations were equivalent to the resulting final concentration of the carrier in each of the test solutions. After it was found in the first group of experiments that carrier concentrations

equivalent to those in the 5 and 1 ppm test solutions did not affect the test polyps only the concentration equivalent to the 10 ppm test solution was used in the control experiments.

The experimental animals were visually examined prior to transfer into the test and control solutions to make sure they were in good condition. In the case of Chrysaora polyps their distribution on the slide was mapped on data sheets to aid in locating them later.

Experimental animals were held in the test solutions for 24 hours. At the end of that period their condition was recorded and the test solutions were replaced with untreated river water. Polyp condition was recorded in respect to the following: contraction state of body and tentacles, body, tentacle and mouth movements, and integrity of body tissues. The test animals were examined every day for a period of six to seven days after termination of the exposure period and their condition recorded. Water was changed in the jars on each day of the post-exposure period. In the case of polyps, especially of Chrysaora on slides, all individuals that survived the tests were held in the same jars after the post-exposure period for as long as they remained alive and in apparent good condition. Once a week water was changed and animals fed brine shrimp.

1. Tests with polyps

Tests with polyps were conducted in 50 ml straight-sided flint glass jars. After the appropriate concentrations had been mixed and poured into each jar a slide with Chrysaora polyps was carefully lowered into each jar avoiding splashing the solution and

making sure the side with polyps was facing up. In the case of Aurelia, individual polyps were transfered into the jars with a bulb pipette.

Numbers of Chrysaora polyps used in any one test solution depended on the number on the particular slide used since set of polyps varied from slide to slide. The numbers used in individual tests ranged from 3 to 34, but in most instances it ranged between 7 and 13. More than one slide was used in a single test solution when needed to raise the number of polyps to the level most frequently used (7 to 13). Five to ten Aurelia polyps were used in most tests.

Most of these polyps had reached the 16-tentacle stage. Many Chrysaora polyps had produced podocysts and in many instances small polyps growing out of these cysts were included in the polyp counts.

During the first tests with polyps the test specimens were examined at different intervals in a geometric progression during the 24-hour exposure period: 0.5, 1, 3, 6, 12 and 24 hours. This way it was possible to detect gradual changes in the condition of polyps when affected by a chemical. However, we could not establish with certainty the time at which a particular polyps died or even whether or not it was definitely dead. Therefore, it was decided to examine them only at the end of 24 hours and to determine mortality on the basis of whether or not a polyp recovered fully during the 6 to 7-day post-exposure period.

The mortality figures presented in the results in effect represent the conditions existing at the end of the post-exposure

period rather than those existing at the end of the 24-hour exposure period.

Generally, the polyps that survived exposure to chemicals also remained alive as long as they were held in untreated river water in the laboratory. Many of these were subjected to tests with another chemical two or three months after the end of their exposure to the first chemical. When thus used in a second test these polyps (termed by us "used" for conveneience) represented replications to identical tests using "new" polyps, i.e., polyps that had not been previously exposed to any chemical. New polyps were used whenever an earlier test was repeated.

2. Tests with ephyrae

Tests with ephyrae were also conducted in 50 ml jars similar to those used in tests with polyps. The number used in each jar ranged from 4 to 20 but in most instances it was between 8 and 14.

3. Tests with medusae

Only two tests were conducted with Chrysaora medusae before tests with polyps were begun.

Four to eight medusae (most frequently four to six) were held in 1/2 gallon flint glass jars containing the test solutions. Their activity was monitored at different time intervals during the 24-hour exposure period but a final decision on death of any one individual was withheld till after the test solutions had been replaced with untreated river water.

Most of the medusae used measured two-to-three inches in bell diameter.

III. RESULTS AND DISCUSSION

Ninety eight compounds were screened for their effect on *Chrysaora quinquecirrha* polyps between 12 August 1970 and 25 March 1971. Seventy-three of these were screened on *Aurelia aurita* polyps during the same period. Besides tests on polyps, 11 of the chemicals were tested on *Aurelia* ephyrae, two on *Chrysaora* medusae and one on *Chrysaora* ephyrae.

Table 1 presents the results of all tests conducted. Summaries extracted from the data on Table 1 appear on Tables 2 and 3 and, with additional information, on Table 4.

A. Mortality tests with polyps

Most of the chemicals tested caused no mortality among the polyps of *Chrysaora* or *Aurelia* (Table 1). Sixty-nine of 95, or 73%, of the chemicals tested at a concentration of 10 PPM caused no mortality on *Chrysaora* polyps. In tests with *Aurelia*, 55 of 69, or 80% of the chemicals caused no mortality at that concentration. (Table 2A).

A higher percentage of the chemicals tested caused no mortality at 5 and 1 PPM than at 10 ppm. At 5 PPM, 78 of 98 (80%) of the chemicals caused no mortality on *Chrysaora* and 60 of 70 (86%) had no effect on *Aurelia*. At 1 PPM, 89 of 95 (94%) chemicals caused no mortality on *Chrysaora* and 41 of 48 (85%) had no effect on *Aurelia* (Table 2A).

Control tests were satisfactory in nearly every test. Mortalities among control animals were recorded in only four instances.

Most of the chemicals had a similar effect on Chrysaora and Aurelia polyps, i.e., they caused no mortality on either species or mortality was about the same for both (Tables 2B and 3). Some chemicals, however, caused different mortalities in the two species. At 10 PPM, 9 chemicals caused mortality on Chrysaora but not on Aurelia; the reverse was true with 3 other chemicals (Table 2A). At 5 PPM, 7 chemicals caused mortality on Chrysaora but not Aurelia while 2 chemicals caused mortality only on Aurelia. At 1 PPM 2 chemicals caused mortality on Chrysaora only while the reverse was true for 3 other chemicals.

Table 3 presents the observed mortalities in polyps of Chrysaora and Aurelia caused by 20 chemicals that produced different mortalities on the two species. Many of the differences are great and in most instances involve zero mortality for Aurelia at concentrations that caused a significant mortality on Chrysaora.

The results presented in the preceding paragraphs point out a definite difference in resistance to certain chemicals between the polyps of Chrysaora and Aurelia. This differential resistance between two very closely related organisms strengthens the possibility of finding a chemical that will cause a significant mortality on Chrysaora polyps without seriously affecting other organisms in the estuarine ecosystem.

We have screened chemicals at concentrations acknowledged to be higher than desired (10 and 5 PPM). This has been done to provide additional data to support whatever conclusions we may reach about the effectiveness of a particular chemical. A chemical that fails to cause a significant mortality among polyps at 10 and 5 PPM may be safely set aside as being useless for control of those organisms.

Our aim is to find chemicals that are effective at the lowest concentrations possible. To date, five of those tested caused mortality at 0.5 PPM. Of those five, two have caused mortality at 0.1 PPM. These five chemicals are: 4',5-Dibromo-3-nitrosalicylanilide, 4'-Iodo-5-nitrosalicylanilide, Malachite green oxalate, Bromsalans and Triphenyltin chloride. Malachite green oxalate and triphenyltin chloride are the only ones that have caused a mortality at 0.1 PPM (Table 4).

Examination of the test polyps in the 0.5 PPM solutions at the end of the 24-hour exposure period showed that the effect of the above chemicals at that concentration (and all other higher concentrations) was accompanied by partial or advanced disintegration of the polyps. We distinguish partial from advanced disintegration in that partially disintegrated polyps are still discernible as polyps in their overall shape and other morphological features while in the advanced state of disintegration only a blob of tissue remained.

Further tests will be conducted with the above chemicals to verify the mechanism by which they cause mortality on polyps. Several other chemicals included in Table 1 will also be tested further. Among these is Tubercidin, a nucleocide antibiotic, which caused 100% mortality on both Chrysaora and Aurelia at a concentration of 1 PPM.

B. Mortality tests with ephyrae

Tests using ephyrae were conducted whenever sufficient numbers of this life-stage were available. Enough were available for 11 tests with Aurelia and one with Chrysaora (Table 1).

The single test with Chrysaora ephyrae was conducted with 3-Trifluormethyl-4-nitrophenol. It resulted in a 50% mortality at 10 PPM and no mortality at 5 and 1 PPM. This chemical also failed to produce mortality on Chrysaora polyps at 10 PPM and lower concentrations.

Eight of the nine chemicals tested at 10 PPM on Aurelia ephyrae caused mortality among them - 100% in five cases. Six of these chemicals and a seventh are not tested at 10 PPM produced mortality at 5 PPM. Six of the latter also caused mortality at 1 PPM. The only two tested at 0.5 PPM, 4',5-Dibromo-3-nitrosalicylanilide and Pentachlorophenol, produced 100% mortality. The only chemical tested at 0.1 PPM was 4',5-Dibromo-3-nitrosalicylanilide and a mortality of 37.5% (3 out of 8) was recorded.

The high percentage of chemicals that caused mortality among Aurelia ephyrae appears to suggest that this life-stage may be more susceptible than the polyp stage to the effect of chemicals. However, most of the chemicals screened with ephyrae were selected on the basis of having caused mortality on polyps in previous tests. Nevertheless, the effect of chemicals tested on both Aurelia polyps and ephyrae support the suggestion that ephyrae may be more susceptible than polyps.

This tests with Pentachlorophenol and Triphenyltin chloride will be repeated since in both cases all control animals also died. Likewide, further tests at lower concentrations will be conducted with 4',5-Dibromo-3-nitrosalicylanilide when more ephyrae are available. Other chemicals will also be tested to whatever extent the supply of ephyrae permits.

C. Mortality tests with medusae

Two tests were conducted with Chrysaora medusae using Catechol and 3-Trifluormethyl-4-nitrophenol. Catechol produced 100% mortality at all four concentrations tested, including 0.5 PPM. This chemical was much more effective on medusae than on polyps. In tests with the latter only partial mortalities were recorded at 10 and 5 PPM and none at 1 or 0.5 PPMΣ

No mortality was observed with 3-Trifluormethyl-4-nitrophenol at 5 and 0.5 PPM.

More tests with medusae will be conducted in the spring and summer of 1971.

IV. CONCLUSION

The series of tests conducted to date have pointed out several compounds that show promise as agents for control of jellyfish in the Chesapeake Bay region by their effect on polyps, ephyrae and medusae. The leads suggested by these results will be followed-up in further tests with the same and related chemicals and in tests with new groups of compounds expected to have an effect on the enzymatic activities of jellyfish.

Further attention will be given to the apparent differences in susceptibility to chemicals between polyps, ephyrae and medusae.

TABLE 1.

JELLYFISH MORTALITY S[illegible]ES (HAVEN AND MORALES)
PERCENT MORTALITY [illegible]TER 24-HR EXPOSURE

SPECIES	CONDITION	CHEM NBR	CHEMICAL	DATE	CHEMICAL CONCENTRATION (PPM) 10	5	1	0.5	0.1	0.05	0.01	CARRIER	CARRIER CONTROL (EQUIVALENT PPM) 10	5	1	WATER CONTROL WC
Chrysaora quinquecirrha POLYPS																
CP	N	9	2-Bromo-4-nitrophenol	100870	0.0	0.0	0.0	0.0				1	0.0	0.0	0.0	0.0
CP	N	35	3-Bromo-4-nitrophenol	121570	0.0	0.0	0.0					1	0.0			0.0
CP	N	8	2-Chloro-4-nitrophenol	100770	0.0	0.0	0.0	0.0				1	0.0	0.0	0.0	0.0
CP	N	11	3-Chloro-4-nitrophenol	101470	0.0	0.0	0.0					0				0.0
CP	N	10	5-Chloro-2-nitrophenol	101370	0.0	0.0	0.0					0				0.0
CP	N	6	2,5-Dichloro-4-nitrophenol	100570	25.0	0.0	0.0	0.0				1	0.0	0.0	0.0	0.0
CP	N	7	3,4,6-Trichloro-2-nitrophenol	100670	0.0	0.0	0.0	0.0				1	0.0	0.0	0.0	0.0
CP	N	2	3-Fluoro-4-nitrophenol	082070	*	0.0						1	0.0	0.0		0.0
CP	N	5	3-Trifluormethly-2-nitrophenol	100270	0.0	0.0	0.0	0.0				1	0.0	0.0	0.0	**
CP	N	1	3-Trifluromethyl-4-nitrophenol	081470	0.0	0.0		0.0				1	0.0	0.0		0.0
CP	N	12	3-Trflrmthyl-4-nitrphnl (Na Salt)	101570	0.0	0.0	0.0					1	0.0			0.0
CP	N	17	3'-Bromo-3-nitrosalicylanilide	112470	72.7	83.3	0.0					1	0.0			0.0
CP	N	17	3'-Bromo-3-nitrosalicylanilide	010871			0.0	0.0	0.0			1	0.0			0.0
CP	N	13	4'-Bromo-3-nitrosalicylanilide	111970	87.5	100.0	0.0					1	0.0			0.0
CP	N	25	4',5-Dibromo-3-nitrosalicylanilide	120170	100.0	100.0	100.0					1	0.0			0.0
CP	N	25	4',5-Dibromo-3-nitrosalicylanilide	010871			58.3	50.0	0.0			1	0.0			0.0
CP	N	21	4'-Bromo-5-nitrosalicylanilide	112770	70.0	71.4	0.0					1	0.0			0.0
CP	N	32	2'-Chloro-3-nitrosalicylanilide	120970	0.0	0.0	0.0					1	0.0			0.0
CP	N	3	3'-Chloro-3-nitrosalicylanilide	082770	73.5	29.6		0.0				1	0.0	0.0		0.0
CP	N	3	3'-Chloro-3-nitrosalicylanilide	111870	100.0	12.5	0.0					1	0.0			0.0
CP	N	28	2'-Chloro-5-nitrosalicylanilide	120270	83.3	0.0	0.0					1	0.0			0.0
CP	N	65	3'-Chloro-5-nitrosalicylanilide	120370	53.8	0.0	0.0					1	0.0			0.0
CP	N	64	4'-Chloro-5-nitrosalicylanilide	120370	100.0	14.2	0.0					1	0.0			0.0
CP	N	63	Isomeric mixture: [1] [2]	120370	0.0	0.0	0.0					1	0.0			0.0
CP	N	30	Isomeric mixture: [3]	120870	55.5	66.6	0.0					1	0.0			0.0
CP	N	18	Synergistic mixture:	112570	66.6	100.0	0.0					1	0.0			0.0
CP	N	14	3'-Fluoro-3-nitrosalicylanilide	111970	100.0	26.6	0.0					1	0.0			0.0
CP	N	23	4'-Fluoro-3-nitrosalicylanilide	120170	0.0	0.0	0.0					1	0.0			0.0
CP	N	34	3'-Iodo-3-nitrosalicylanilide	120970	33.3	0.0	0.0					1	0.0			0.0
CP	N	24	4'-Iodo-3-nitrosalicylanilide	120170	100.0	85.7	0.0					1	0.0			0.0
CP	N	31	4'-Iodo-5-nitrosalicylanilide	120870	100.0	100.0	100.0					1	0.0			0.0
CP	N	31	4'-Iodo-5-nitrosalicylanilide	010871		100.0	100.0	42.8	0.0			1	0.0			0.0
CP	N	15	3-Nitrosalicylic Acid	112470	0.0	0.0	0.0					1	0.0			0.0
CP	N	16	3-Nitrosalicoyl cl	112470	0.0	0.0	0.0					1	0.0			0.0
CP	N	33	3,5-Dinitrosalicylic Acid	120970	0.0	0.0	0.0					1	0.0			0.0
CP	N	20	Propioaniline	112570	0.0	0.0	0.0					1	0.0			0.0
CP	N	29	p-Bromoaniline	120270	0.0	0.0	0.0					1	0.0			0.0
CP	N	26	p-Iodoaniline	120270	0.0	0.0	0.0					1	0.0			0.0
CP	N	19	2,4,6-Trichloroaniline	112570	0.0	0.0	0.0					1	0.0			0.0
CP	N	27	5-Ethl-6-nitr-phnl-met-thiaz-2,4-d [4] [5]	120270	0.0	0.0	0.0					1	0.0			0.0
CP	N	22	5-Ethl-6-phenl-met-thiaz-2,4-dione	112770	0.0	0.0	0.0					1	0.0			0.0
CP	N	4	Catechol	090470	50.0	50.0	0.0	0.0				0				0.0
CP	N	4	Catechol	100170	59.9	4.4	0.0	0.0				0				0.0

TABLE I - Continued

JELLYFISH MORTALITY STUDI (HAVEN AND MORALES)
PERCENT MORTALITY AFTER 24-HR EXPOSURE

SPECIES	CONDITION	CHEMICAL NUMBER	CHEMICAL	DATE	CHEMICAL CONCENTRATION (PPM) 10	5	1	0.5	0.1	0.05	0.01	CARRIER CONTROL (EQUIVALENT PPM) CARRIER	10	5	1	WATER CONTROL WC
Chrysaora quinquecirrha POLYPS																
CP	N	38	Pyrocatechol	121570	23.0	0.0	0.0					2	0.0	0.0	0.0	0.0
CP	N	39	Tetraphenylarsonium bromide	121670	0.0	0.0	0.0					2	0.0	0.0	0.0	0.0
CP	N	36	Tetraphenylarsonium chloride	121570	0.0	0.0	0.0					1	0.0			0.0
CP	N	37	Tetra	121570	0.0	0.0	0.0					1	0.0			0.0
CP	N	40	Sodium tetrathionate	121670	0.0	0.0	0.0					2	0.0	0.0	0.0	0.0
CP	N	92	p-Aminophenol	032571	93.7	59.9	0.0					2	0.0			0.0
CP	U	92	p-Aminophenol	032571	100.0	68.1	0.0					2	0.0			0.0
CP	N	91	p-Aminoazobenzene	032571	0.0	0.0	0.0					2	0.0			0.0
CP	U	91	p-Aminoazobenzene	032571	0.0	0.0	0.0					2	0.0			0.0
CP	N	94	Alizarin Yellow R	032471	0.0	0.0	0.0					2	0.0			0.0
CP	U	94	Alizarin Yellow R	032471	0.0	0.0	0.0					2	0.0			0.0
CP	N	87	Acetonitrile	031871	0.0	0.0	0.0					0				0.0
CP	U	87	Acetonitrile	031871	0.0	0.0	0.0					0				0.0
CP	N	88	Acetanilide	031871	0.0	0.0	0.0					0				0.0
CP	U	88	Acetanilide	031871	0.0	0.0	0.0					0				0.0
CP	N	89	Acetaldoxime	031871	0.0	0.0	0.0					0				0.0
CP	U	89	Acetaldoxime	031871	0.0	0.0	0.0					0				0.0
CP	N	98	Acetamide	032371	0.0	0.0	0.0					2	0.0			0.0
CP	U	98	Acetamide	032371	0.0	0.0	0.0					2	0.0			0.0
CP	N	97	Acetal	032371	0.0	0.0	0.0					2	0.0			0.0
CP	U	97	Acetal	032371	0.0	0.0	0.0					2	0.0			0.0
CP	N	96	Abietic Acid	032371	0.0	0.0	0.0					2	0.0			0.0
CP	U	96	Abietic Acid	032371	0.0	0.0	0.0					2	0.0			0.0
CP	N	48	2,3-Dichloro-1,4-naphthoquinone	012071	100.0	100.0	0.0					1	0.0			0.0
CP	N	48	2,3-Dichloro-1,4-naphthoquinone	012771			0.0	0.0	0.0			1	0.0			0.0
CP	N	90	2-Aminoanthraquinone	032571	0.0	0.0	0.0					2	0.0			0.0
CP	U	90	2-Aminoanthraquinone	032571	0.0	0.0	0.0	0.0				2	0.0			0.0
CP	N	73	Quinoline	020471	0.0	0.0	0.0					2	0.0			0.0
CP	U	73	Quinoline	020471	0.0	0.0	0.0					2	0.0			0.0
CP	N	43	2-Chloroquinoline	121970	0.0	0.0						2	0.0	0.0	0.0	0.0
CP	N	68	1,2,3,4-Tetrahydroquinoline	020371	0.0	0.0	0.0					2	0.0			0.0
CP	U	68	1,2,3,4-Tetrahydroquinoline	020371	0.0	0.0	0.0					2	0.0			0.0
CP	N	75	3-Aminoquinoline	020971	0.0	0.0	0.0					1	0.0			0.0
CP	U	75	3-Aminoquinoline	020971	0.0	0.0	0.0					1	0.0			0.0
CP	N	60	Tetramethylurea	020271	0.0	0.0	0.0					2	0.0			0.0
CP	U	60	Tetramethylurea	020271	0.0	0.0	0.0					2	0.0			0.0
CP	N	41	1-Acetyl-2-phenylhydrazine	121970	0.0	0.0	0.0					2	0.0	0.0	0.0	0.0
CP	N	45	1-Acetyl-2-phenylhydrazine	011471		0.0	0.0	0.0				2		0.0		0.0
CP	N	62	Pyridazine	020271	0.0	0.0	0.0					2	0.0			0.0
CP	U	62	Pyridazine	020271	0.0	0.0	0.0					2	0.0			0.0
CP	N	69	Pyridine	020371	0.0	0.0	0.0					2	0.0			0.0
CP	U	69	Pyridine	020371	0.0	0.0	0.0					2	0.0			0.0
CP	N	66	1,2,5,6-Tetrahydropyridine	020371	0.0	0.0	0.0					2	0.0			0.0

TABLE I - Continued

JELLYFISH MORTALITY STUDY (HAVEN AND MORALES)
PERCENT MORTALITY AFTER 24-HR EXPOSURE

SPECIES	CONDITION	CHEMICAL NUMBER	CHEMICAL	DATE	CHEMICAL CONCENTRATION (PPM) 10	5	1	0.5	0.1	0.05	0.01	CARRIER CONTROL (EQUIVALENT PPM) 10	5	1	WATER CONTROL WC
Chrysaora quinquecirrha POLYPS															
CP	U	66	1,2,5,6-Tetrahydropyridine	020371	0.0	0.0	0.0					2 0.0			0.0
CP	N	74	Butonate	020971	0.0	0.0	0.0					1 0.0			0.0
CP	U	74	Butonate	020971	0.0	0.0	0.0					1 0.0			0.0
CP	N	67	n-Butyl propionate	020371	0.0	0.0	0.0					2 0.0			0.0
CP	U	67	n-Butyl propionate	020371	0.0	0.0	0.0					2 0.0			0.0
CP	N	76	n-Butyl chloride	020971	0.0	0.0	0.0					2 0.0			0.0
CP	U	76	n-Butyl chloride	020971	0.0	0.0	0.0					2 0.0			0.0
CP	N	59	Tert-butyl chloride	020271	0.0	0.0	0.0					2 0.0			0.0
CP	U	59	Tert-butyl chloride	020271	0.0	0.0	0.0					2 0.0			0.0
CP	N	61	Propyl chloride	020271	0.0	0.0	0.0					2 0.0			0.0
CP	U	61	Propyl chloride	020271	0.0	0.0	0.0					2 0.0			0.0
CP	N	42	2,4,5-Trichlorophenoxypropionic Acid	121970	0.0	0.0	0.0					2 0.0	0.0	0.0	0.0
CP	N	51	2-(2',4',5',-Trichlphnoxy) prop ac.[6]	012071	0.0	0.0	0.0					2 0.0			0.0
CP	N	52	Abate	012171	0.0	0.0	0.0					2 0.0			0.0
CP	N	53	Bromsalans	012171	100.0	100.0	100.0					1 0.0			0.0
CP	N	53	Bromsalans	012771			100.0	100.0	0.0			1 0.0			0.0
CP	N	54	Amidithion	012171	0.0	0.0	0.0					1 0.0			0.0
CP	N	55	C-9491	012171	100.0	100.0	0.0					1 0.0			0.0
CP	N	57	C-9491 Oxygen analog	012871	0.0	0.0	0.0					1 0.0			0.0
CP	N	56	Ametryne	012871	0.0	0.0	0.0					1 0.0			0.0
CP	N	58	Atratone	012871	0.0	0.0	0.0					1 0.0			0.0
CP	N	70	Bromophos-ethyl	020471	0.0	0.0	0.0					1 0.0			0.0
CP	U	70	Bromophos-ethyl	020471	0.0	0.0	0.0					1 0.0			0.0
CP	N	71	Carbophenothion	020471	0.0	0.0	0.0					1 0.0			0.0
CP	U	71	Carbophenothion	020471	0.0	0.0	0.0					1 0.0			0.0
CP	N	72	DDVP	020471	0.0	0.0	0.0					1 0.0			0.0
CP	U	72	DDVP	020471	0.0	0.0	0.0					1 0.0			0.0
CP	N	50	Malachite green oxalate	012071	100.0	100.0	100.0					2 0.0			0.0
CP	N	50	Malachite green oxalate	012771			100.0	100.0	83.3			2		0.0	0.0
CP	N	49	1,2,3,4,5,6-Hexachlorocylohexane	012071	0.0	0.0	0.0					1 0.0			0.0
CP	N	47	Pentachlorophenol	011471		0.0	0.0	0.0				2	0.0		0.0
CP	N	47	Pentachlorophenol	012871	63.6	0.0	0.0					2 0.0			0.0
CP	N	46	Triphenyltin chloride	011471	100.0	100.0	100.0					2	0.0		0.0
CP	N	46	Triphenyltin chloride	012771			100.0	100.0	100.0			2		0.0	0.0
CP	N	44	p-Aminoacetophenone	011471		0.0	0.0	0.0				2	0.0		0.0
CP	N	95	m-Aminoacetophenone	032471	0.0	0.0	0.0					2 0.0			0.0
CP	U	95	m-Aminoacetophenone	032471	0.0	0.0	0.0					2 0.0			0.0
CP	N	93	Acetophenone	032471	0.0	0.0	0.0					2 0.0			0.0
CP	U	93	Acetophenone	032471	0.0	0.0	0.0					2 0.0			0.0
CP	N	78	Dihydrouracil	030971	0.0	0.0	0.0					2 0.0			0.0
CP	U	78	Dihydrouracil	030971	0.0	0.0	0.0					2 0.0			0.0
CP	N	84	Adenosine	031671	0.0	0.0	0.0					2 0.0			0.0
CP	U	84	Adenosine	031671	0.0	0.0	0.0					2 0.0			0.0

TABLE I - Continued

JELLYFISH MORTALITY STUDIES (HAVEN AND MORALES)
PERCENT MORTALITY AFTER 24-HR EXPOSURE

SPECIES	CONDITION	CHEM NBR	CHEMICAL	DATE	CHEMICAL CONCENTRATION (PPM) 10	5	1	0.5	0.1	0.05	0.01	CARRIER NBR	CARRIER CONTROL (EQUIVALENT PPM) 10	5	1	WATER CONTROL WC
Chrysaora quinquecirrha POLYPS																
CP	N	85	Dexyadenosine	031671	0.0	0.0	0.0					2	0.0			0.0
CP	U	85	Dexyadenosine	031671	0.0	0.0	0.0					2	0.0			0.0
CP	N	86	Tubercidin	031771	100.0	100.0	100.0					2	0.0			0.0
CP	U	86	Tubercidin	031771	100.0	100.0	100.0					2	0.0			0.0
CP	N	83	Uridine	031671	0.0	0.0	0.0					2	0.0			0.0
CP	U	83	Uridine	031671	0.0	0.0	0.0					2	0.0			0.0
CP	N	81	6-Azauridine	031071	0.0	0.0	0.0									0.0
CP	U	81	6-Azauridine	031071	0.0	0.0	0.0					2	0.0			0.0
CP	N	79	5-Bromouridine	030971	0.0	0.0	0.0					2	0.0			0.0
CP	U	79	5-Bromouridine	030971	0.0	0.0	0.0					2	0.0			0.0
CP	N	80	5-Chlorouridine	031071	0.0	0.0	0.0									0.0
CP	U	80	5-Chlorouridine	031071	0.0	0.0	0.0					2	0.0			0.0
CP	N	77	5-Iodouridine	030971	0.0	0.0	0.0					2	0.0			0.0
CP	U	77	5-Iodouridine	030971	0.0	0.0	0.0					2	0.0			0.0
CP	N	82	Pseudouridine	031071	100.0	100.0	0.0									0.0
CP	U	82	Pseudouridine	031071	100.0	100.0	0.0					2	100.0***			
Chrysaora quinquecirrha MEDUSAE																
CM	N	4	Catechol	090370	100.0	100.0	100.0	100.0				0				0.0
CM	N	1	3-Trifluoromethly-4-nitrophenol	081270		0.0				0.0		1	0.0			0.0
Chrysaora quinquecirrha EPHYRAE																
CE	N	1	3-Trifluormethyl-4-nitrophenol	081470	50.0	0.0		0.0				1	0.0			0.0
Aurelia aurita POLYPS																
AP	N	35	3-Bromo-4-nitrophenol	121570	0.0	0.0						0				0.0
AP	N	17	3'-Bromo-3-nitrosalicylanilide	112470	0.0	0.0	0.0					1	0.0			0.0
AP	N	13	4'-Bromo-3-nitrosalicylanilide	111970	0.0	0.0	0.0					1	0.0			0.0
AP	N	25	4',5-Dibromo-3-nitrosalicylanilide	120170	100.0	83.3						0				0.0
AP	N	29	4',5-Dibromo-3-nitrosalicylanilide	010871			0.0	0.0	0.0			1	0.0			0.0
AP	N	21	4'-Bromo-5-nitrosalicylanilide	112770	100.0							0	0.0			0.0
AP	N	32	2'-Chloro-3-nitrosalicylanilide	120970	0.0	0.0						0				0.0
AP	N	28	2'-Chloro-5-nitrosalicylanilide	120270	0.0	0.0						0				0.0
AP	N	65	3'-Chloro-5-nitrosalicylanilide	120371	0.0	0.0						0				0.0
AP	N	64	4'-Chloro-5-nitrosalicylanilide	120371	0.0	0.0						0				0.0
AP	N	63	Isomeric mixture:[1]	120371	0.0	0.0						0				0.0
AP	N	30	Isomeric mixture:[2]	120870	0.0	0.0						0				0.0

TABLE I - Continued

JELLYFISH MORTALITY STUDIES (HAVEN AND MORALES)
PERCENT MORTALITY AFTER 24-HR EXPOSURE

SPECIES	CONDITION	CHEMICAL NUMBER	CHEMICAL	DATE	CHEMICAL CONCENTRATION (PPM) 10	5	1	0.5	0.1	0.05	0.01	CARRIER CONTROL (EQUIVALENT PPM) CARRIER	10	5	1	WATER CONTROL WC
Aurelia aurita POLYPS																
AP	N	14	3'-Fluoro-3-nitrosalicylanilide	111970	0.0	0.0	0.0					1	0.0			0.0
AP	N	23	4'-Fluoro-3-nitrosalicylanilide	120170	0.0	0.0	0.0					0				0.0
AP	N	34	3'-Iodo-3-nitrosalicylanilide	120970	0.0	0.0						0				0.0
AP	N	24	4'-Iodo-3-nitrosalicylanilide	120170	0.0	0.0						0				0.0
AP	N	31	4'-Iodo-5-nitrosalicylanilide	120870	80.0	0.0						0				0.0
AP	N	15	3-Nitrosalicylic Acid	112470	0.0	0.0	0.0					1	0.0			0.0
AP	N	16	3-Nitrosalicoyl cl	112470	0.0	0.0	0.0					1	0.0			0.0
AP	N	33	3,5-Dinitrosalicylic Acid	120970	0.0	0.0						0				0.0
AP	N	29	p-Bromoaniline	120870	0.0	0.0						0				0.0
AP	N	26	p-Iodoaniline	120270	0.0	0.0						0				0.0
AP	N	27	5-Ethl-6-nitr-phnl-met-thiaz-2,4-d[4]	120270	0.0	0.0						0				0.0
AP	N	22	5-Ethl-6-phenl-met-thiaz-2,4-dione[5]	112770	0.0							0	0.0			0.0
AP	N	38	Pyrocatechol	121670	0.0	0.0						2	0.0	0.0	0.0	0.0
AP	N	39	Tetraphenylarsonium bromide	121670	100.0	57.1						2	0.0	0.0	0.0	0.0
AP	N	36	Tetraphenylarsonium chloride	121570	0.0	0.0						0				0.0
AP	N	37	Tetraethylammonium chloride	121570	0.0	0.0						0				0.0
AP	N	40	Sodium tetrathonate	121670	0.0	0.0						2	0.0	0.0	0.0	0.0
AP	N	92	p-Aminophenol	032571	100.0	100.0	25.0					2	0.0			0.0
AP	N	91	p-Aminoazobenzene	032571	0.0	0.0	0.0					2	0.0			0.0
AP	N	94	Alizarin Yellow R	032471	0.0	0.0	0.0					2	0.0			0.0
AP	N	87	Acetonitrile	031871	0.0	0.0	0.0					0				0.0
AP	N	88	Acetanilide	031871	0.0	0.0	0.0					0				0.0
AP	N	89	Acetaldoxime	031871	0.0	0.0	0.0					0				0.0
AP	N	97	Acetal	032371	0.0	0.0	0.0					2	0.0			0.0
AP	N	98	Acetamide	032371	0.0	0.0	0.0					2	0.0			0.0
AP	N	96	Abietic Acid	032371	0.0	0.0	0.0					2	0.0			0.0
AP	N	48	2,3-Dichloro-1,4-naphthoquinone	012071	100.0	100.0	100.0					1	0.0			0.0
AP	N	48	2,3-Dichloro-1,4-naphthoquinone	012771			60.0	0.0				1	59.9			0.0
AP	N	90	2-Aminoanthraquinone	032571	0.0	0.0	0.0					2	0.0			0.0
AP	N	43	2-Chloroquinoline	121970	10.0							2	0.0	0.0	0.0	0.0
AP	N	68	1,2,3,4-Tetrahydroquinoline	020371	0.0	0.0	0.0					2	0.0			0.0
AP	N	75	3-Aminoquinoline	020971	0.0	0.0	0.0					1	0.0			0.0
AP	N	60	Tetramethylurea	020271	0.0	0.0	0.0					2	0.0			0.0
AP	N	41	1-Acetyl-2-phenylhydrazine	121970	0.0	0.0						2	0.0	0.0	0.0	0.0
AP	N	45	2,5-Piperazinedione	011471		0.0	0.0	0.0				2		0.0		0.0
AP	N	62	Pyridazine	020271	0.0	0.0	0.0					2	0.0			0.0
AP	N	69	Pyridine	020371	0.0	0.0	0.0					2	0.0			0.0
AP	N	66	1,2,5,6-Tetrahydropyridine	020371	0.0	0.0	0.0					2	0.0			0.0
AP	N	74	Butonate	020971		0.0	0.0					1	0.0			0.0
AP	N	67	n-Butyl propionate	020371	0.0	0.0	0.0					2	0.0			0.0
AP	N	76	n-Butyl chloride	020971		0.0						2	0.0			0.0
AP	N	59	Tert-butyl chloride	020271	0.0	0.0	0.0					2	0.0			0.0
AP	N	61	Propyl chloride	020271	0.0	0.0	0.0					2	0.0			0.0

TABLE I - Continued

JELLYFISH MORTALITY STUDIES (HAVEN AND MORALES)
PERCENT MORTALITY AFTER 24-HR EXPOSURE

SPECIES	CONDITION	CHEMICAL NUMBER	CHEMICAL	DATE	CHEMICAL CONCENTRATION (PPM) 10	5	1	0.5	0.1	0.05	0.01	CARRIER	CARRIER CONTROL (EQUIVALENT PPM) 10	5	1	WATER CONTROL WC
Aurelia aurita POLYPS																
AP	N	42	2,4,5-Trichlorophenoxypropionic Acid	121970	0.0	0.0						2	0.0	0.0	0.0	0.0
AP	N	51	2-(2',4',5',-Trichlphnoxy) prop ac[6]	012071	0.0	0.0	0.0					2	0.0			0.0
AP	N	52	Abate	012171	0.0	0.0	0.0					1	0.0			0.0
AP	N	53	Bromsalans	012171	100.0	66.6	25.0					1	0.0			0.0
AP	N	53	Bromsalans	012771			20.0	0.0	0.0			1			59.9	0.0
AP	N	54	Amidithion	012171	0.0	0.0	0.0					1	0.0			0.0
AP	N	55	C-9491	012171	100.0	100.0	0.0					1	0.0			0.0
AP	N	57	C-9491 Oxygen analog	012871	100.0	0.0	0.0					1	0.0			0.0
AP	N	56	Ametryne	012871	0.0	0.0	0.0					1	0.0			0.0
AP	N	58	Atratone	012871	0.0	0.0	0.0					1	0.0			0.0
AP	N	50	Malachite green oxalate	012071	100.0	100.0	100.0					2	0.0			0.0
AP	N	50	Malachite green oxalate	012771			100.0	100.0	20.0			2			0.0	0.0
AP	N	49	1,2,3,4,5,6-Hexachlorocyclohexane	012071	0.0	0.0	0.0					1	0.0			0.0
AP	N	47	Pentachlorophenol	011471		0.0	14.2	0.0				2		0.0		0.0
AP	N	47	Pentachlorophenol	012871	100.0	0.0	0.0					2	0.0			0.0
AP	N	46	Triphenyltin chloride	011471	100.0	100.0	100.0					2		0.0		0.0
AP	N	46	Triphenyltin chloride	012771			100.0	100.0	100.0			2			0.0	0.0
AP	N	44	p-Aminoacetophenone	011471		0.0	0.0	0.0				2		0.0		0.0
AP	N	95	m-Aminoacetophenone	032471	0.0	0.0	0.0					2	0.0			0.0
AP	N	93	Acetophenone	032471	0.0	0.0	0.0					2	0.0			0.0
AP	N	84	Adenosine	031671	0.0	0.0	0.0					2	0.0			0.0
AP	N	85	Deoxyadenosine	031671	0.0	0.0	0.0					2	0.0			0.0
AP	N	86	Tubercidin	031771	100.0	100.0	100.0					2	0.0			0.0
AP	N	83	Uridine	031671	0.0	0.0	0.0					2	0.0			0.0
Aurelia aurita EPHYRAE																
AE	N	10	5-Chloro-2-nitrophenol	010571	37.5	0.0	0.0					0				0.0
AE	N	13	4'-Bromo-3-nitrosalicylanilide	010671	100.0	80.0	77.7					1	0.0	0.0	0.0	0.0
AE	N	25	4',5-Dibromo-3-nitrosalicylanilide	010571	100.0	100.0	100.0	100.0	37.5			0				0.0
AE	N	21	4'-Bromo-5-nitrosalicylanilide	010671	100.0	100.0	100.0					1	0.0	0.0	0.0	0.0
AE	N	32	2'-Chloro-3-nitrosalicylanilide	010671	87.5	0.0	0.0					1	0.0	0.0	0.0	0.0
AE	N	3	3'-Chloro-3-nitrosalicylanilide	010571	100.0	100.0	81.2					0				0.0
AE	N	4	Catechol	010571	87.5	85.7	0.0					0				0.0
AE	N	45	2,5-Piperazinedione	011471	0.0	0.0	0.0					2		0.0		0.0
AE	N	47	Pentachlorophenol	011471		100.0	100.0	100.0				2		100.0		100.0
AE	N	46	Triphenyltin chloride	011471	100.0	100.0	100.0					2		100.0		100.0
AE	N	44	p-Aminoacetophenone	011471		0.0	0.0	0.0				2		0.0		0.0
					0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0
					0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0
					0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0
					0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0

TABLE I - CONCLUDED

JELLYFISH MORTALITY STUD (HAVEN AND MORALES),
PERCENT MORTALITY AFTER 24-HR EXPOSURE

KEYS TO SYMBOLS

CONDITION: N = "New" polyps

U = "Used" polyps

CARRIER: 0 = Distilled water

1 = Acetone

2 = Ethanol

FOOTNOTES

* Concentration mixed wrong. However, no mortality was recorded at 25 PPM.

** Used distilled water by mistake. Mortality was 100 %.

*** Ethanol concentration 10 times higher than usual in this test because original stock concentration was lower than usual (50 PPM).

1. Isomeric mixture: 3'-Chloro-5-nitrosalicylanilide
   3'-Chloro-3-nitrosalicylanilide

2. Isomeric mixture: 4'-Chloro-4-nitrosalicylanilide
   4'-Chloro-3-nitrosalicylanilide

3. Synergistic mixture: 3'-Chloro-5-nitrosalicylanilide
   4'-Chloro-5-nitrosalicylanilide
   4'-Chloro-3-nitrosalicylanilide
   3'-Chloro-3-nitrosalicylanilide

4. 5-Ethyl-6-nitro-phenyl-meta-thiazane-2,4-dione

5. 5-Ethyl-6-phenyl-meta-thiazane-2,4-dione

6. 2-(2',4',5',-Trichlorophenoxy) propionic acid

TABLE 2

Number of chemicals causing specified effect in mortality experiments conducted on both Chrysaora quinquecirrha and Aurelia aurita, November 1970-March 1971.

Figures in parentheses represent number of chemicals included.

A-

	Concentration (PPM)				
	10	5	1	0.5	0.1
Effect	(71)	(70)	(47)	(8)	(3)
No mortality in either species	45	53	38	4	1
Similar mortality in both species	10	6	3	2	1
Mortality in C. quinquecirrha only	9	7	2	2	1
Mortality in A. aurita only	3	2	3	0	0

B-

Effect	C. quinquecirrha	A. aurita
No mortality at:		
10.0 PPM	69 (95)	55 (69)
5.0 PPM	78 (98)	60 (70)
1.0 PPM	89 (95)	41 (48)
0.5 PPM	15 (20)	6 (8)
0.1 PPM	5 (7)	2 (4)

TABLE 3

Mortality among Chrysaora quinquecirrha (C) and Aurelia aurita (A) polyps subjected to similar concentrations of chemicals for a 24-hour period in the laboratory.

CHEMICAL	DATE	SPECIES	CONCENTRATION (PPM) 10	5	1	0.5	0.1
3'-Bromo-3-nitrosalicylanilide	24 Nov 1970	C	72.7	83.3	0.0		
		A	0.0	0.0	0.0		
4'-Bromo-3-nitrosalicylanilide	19 Nov 1970	C	87.5	100.0	0.0		
		A	0.0	0.0	0.0		
4'-Bromo-5-nitrosalicylanilide	27 Nov 1970	C	70.0	71.4	0.0		
		A	100.0	----	----		
4',5-Dibromo-3-nitrosalicylanilide	1 Dec. 1970	C	100.0	100.0	100.0		
		A	100.0	83.3	---		
	8 Jan 1971	C			58.3	50.0	0.0
		A			0.0	0.0	0.0
2'-Chloro-5-nitrosalicylanilide	2 Dec 1970	C	83.3	0.0	0.0		
		A	0.0	0.0	---		
3'-Chloro-5-nitrosalicylanilide	3 Dec 1970	C	53.8	0.0	0.0		
		A	0.0	0.0	---		
4'-Chloro-5-nitrosalicylanilide	3 Dec 1970	C	100.0	14.2	0.0		
		A	0.0	0.0	---		
Isomeric mixt.: 4'-Chloro-4-nitrosalicylanilide 4'-Chloro-3-nitrosalicylanilide	8 Dec 1970	C	55.5	66.6	0.0		
		A	0.0	0.0	---		
3'-Fluoro-3-nitrosalicylanilide	19 Nov 1970	C	100.0	26.6	0.0		
		A	0.0	0.0	0.0		
3'-Iodo-3-nitrosalicylanilide	9 Dec 1970	C	33.3	0.0	0.0		
		A	0.0	0.0	0.0		
4'-Iodo-3-nitrosalicylanilide	1 Dec 1970	C	100.0	85.7	0.0		
		A	0.0	0.0	---		
4'-Iodo-5-nitrosalicylanilide	8 Dec 1970	C	85.7	83.3	0.0		
		A	60.0	0.0	---		
Pyrocatechol	15 Dec 1970	C	23.0	0.0	0.0		
		A	0.0	0.0	---		
Tetraphenylarsonium bromide	16 Dec 1970	C	0.0	0.0	0.0		
		A	100.0	57.1	---		
p-Aminophenol	25 Mar 1971	C	93.7	59.9	0.0		
		A	100.0	100.0	25.0		
2.3-Dichloro-1,4-naphthoquinone	23 Mar 1971	C	100.0	100.0	0.0		
		A	100.0	100.0	100.0		
2-Chloroquinoline	19 Dec 1970	C	0.0	0.0	---		
		A	10.0	---	---		
Bromsalans	21 Jan 1971	C	100.0	100.0	100.0		
		A	100.0	66.6	25.0		
	27 Jan 1971	C			100.0	100.0	0.0
		A			20.0	0.0	0.0
C-9491, Oxygen analog	28 Jan 1971	C	0.0	0.0	0.0		
		A	100.0	0.0	0.0		
Malachite Green Oxalate	20 Jan 1971	C	100.0	100.0	100.0		
		A	100.0	100.0	100.0		
	27 Jan 1971	C			100.0	100.0	83.3
		A			100.0	100.0	20.0

TABLE 4

Results of tests that showed mortality of Chrysaora quinquecirrha polyps at a concentration of 0.5 PPM.

Chemical	Date	CONCENTRATION (PPM)								
		1.0			0.5			0.1		
		% Mort.	No. polyps in test	Effect after 24 Hrs.	% Mort.	No. polyps in test	Effect after 24 Hrs.	% Mort.	No. polyps in test	Effec after 24 Hrs.
4',5-Dibromo-3-nitrosalicylanilide	8 Jan 1971	58.3	12	*	50.0	16	*	0.0	11	x
4'-Iodo-5-nitrosalicylanilide	8 Jan 1971	100.0	5	*	42.8	7	*	0.0	9	x
Malachite green oxalate	27 Jan 1971	100.0	13	*	100.0	11	*	83.3	12	**
Bromsalans	27 Jan 1971	100.0	19	**	100.0	6	**	0.0	6	x
Triphenyltin chloride	27 Jan 1971	100.0	9	*	100.0	9	*	100.0	4	*

* = Polyps appear to be in an advanced state of disintegration

** = Polyps partially disintegrated (usually the tentacles).

x = No harmful effect evident

Table 5. Data on nematocysts from medusae of Cyanea capillata.

	"a" atrichs	"A" atrichs	" " atrichs	Holotrichs	Euryteles
No. examined	10	10	10	10	10
length (u)	6.9-10.7	15.8-20.8	6.6-10.9	11.8-14.5	12.7-15.4
width(u)	4.6-6.2	8.0-14.0	2.9-5.6	9.1-11.9	8.3-9.2
mean length (u)	8.6	18.7	8.3	13.3	13.7
mean width (u)	5.2	11.3	3.9	10.7	8.8
greatest L:W ratio	1.76:1	2.95:1	2.55:1	1.31:1	1.73:1
smallest L:W ratio	1.52:1	1.44:1	1.93:1	1.18:1	1.46:1
mean L:W ratio	1.62:1	1.70:1	2.20:1	1.25:1	1.55:1

Table 6. Data on nematocysts from medusae of Rhopilema verrilli.

	"a" atrichs	Holotrichs	Euryteles
No. examined	30	30	30
length (u)	5.3-6.9	6.9-8.9	7.6-10.1
width (u)	3.3-4.5	5.4-7.4	5.0-7.1
mean length (u)	6.2	8.1	9.3
mean width (u)	4.0	6.8	6.1
greatest L:W ratio	1.80:1	1.42:1	1.68:1
smallest L:W ratio	1.45:1	1.09:1	1.32:1
mean L:W ratio	1.59:1	1.20:1	1.51:1

Table 7. Mean lengths and widths of the three nematocyst types in six specimens of Rhopilema verrilli.

Specimen	I	II	III	IV	V	VI
"a" atrichs						
$\bar{x}$ length (u)	6.0	6.0	6.1	6.2	6.6	6.2
$\bar{x}$ width (u)	4.0	3.9	4.0	3.9	4.2	3.7
Holotrichs						
$\bar{x}$ length (u)	8.2	8.0	8.0	8.0	8.0	8.4
$\bar{x}$ width (u)	6.9	6.6	6.5	6.8	6.8	6.9
Euryteles						
$\bar{x}$ length (u)	9.5	8.6	9.3	9.8	9.4	8.9
$\bar{x}$ width (u)	6.1	5.7	6.4	6.4	6.4	5.7

Table 8. Comparison of the nematocysts in medusae of *Aurelia*, *Chrysaora*, *Cyanea* and *Rhopilema* from Chesapeake Bay.

	Aurelia	*Chrysaora*	*Cyanea*	*Rhopilema*
Atrichous isorhizas				
"a" atrichs	+	+	+	+
"A" atrichs		+	+	
" " atrichs	+		+	
Holotrichous haplonemes		+	+	+
Microbasic euryteles	+	+	+	+

JELLYFISH BIOCHEMISTRY

I. Introduction

The comparative biochemical studies of the Chesapeake Bay jellyfish (Phylum Cnidaria, class Scyphozoa) which were initiated by Schmidt and Joseph (Schmidt, R. and J. Joseph, 1971, manuscript in preparation) have been continued. A very promising direction of their work was the quantitation of the lipid components of the jellyfish medusae of Aurelia aurita, Chrysaora quinquecirrha, and Cyanea capillata.

Techniques and quantitative procedures which Schmidt and Joseph employed were (1) silicic acid column chromatography, (2) thin-layer chromatography, (3) infrared spectroscopy, and (4) gas-liquid chromatography. Although much material has been isolated to date, especially of the medusae, their studies required large quantities of material (i.e., 0.05-1.0 gm).

We have extended these studies, particularly with respect to the use of microgram quantities of material. With analytical capabilities at this level of detection, determinations on a few organisms have been pursued, thus obviating the requirement to use large numbers of organisms per determination. We prefer to use 1-4 organisms in an assay rather than 50-100 organisms.

With quantitative measurements of the major classes of biochemicals, the metabolic and developmental mechanisms of polymorphism may be pursued. An understanding of such processes is essential for the rational investigation of growth and development inhibiting substances.

II. Assay Procedures

Although we wish to use several procedures at the sub-microgram level of detection for the study of jellyfish polymorphs, standard colorimetric techniques which are usually sensitive to approximately 10-100 micrograms are currently employed. We wish to quantitate the major classes of compounds which collectively constitute a jellyfish polymorphic cell. These measurements will aid us in understanding environmental conditions to which the polymorphs are exposed. Although these measurements may constitute a final set of data by themselves, they will serve as key aids for evaluating metabolic and growth parameters. Such measurements by themselves provide a static or momentary picture of the dynamic life processes. However, these data are needed for (1) measuring the rates of growth and turnover of components, (2) relating metabolic processes for comparative purposes, (3) providing a base on which to relate effects of stimulators or inhibitors of living processes, and (4) providing the concentration parameters which are essential parts for the design of metabolic experiments.

The classes of sub-cellular components presently measured are listed in Table I.

The procedures for the isolation and separation of non-polar lipids have been described in previous annual reports and elsewhere (Schmidt and Joseph, manuscript in preparation, Joseph et al, 1971). Briefly, extraction of the lipids is obtained by MeOH-Ether-$CHCl_3$ and separations obtained with thin-layer chromatography (Silica Gel G with Hexane: Ether: Acetic Acid - 80:20:1).

Fatty acid methyl esters are prepared by transesterification using BF_3/methanol. Gas-liquid chromatography is carried out

using an SE-30 (3%) column, oven temperature starting at 180° C, and detection by flame-ionization.

III. Polyp Studies

The biochemical studies using polyps fall into 2 main sub-groups:

1) Non-Polar Lipid Studies - a continuation of biochemical studies initiated during previous contract periods.

2) Quantitation of Sub-cellular Components - the re-orientation of biochemical studies with eventual emphasis on metabolic events of single polyps.

1. Non-Polar Lipid Studies

Using 2-4 polyps of Aurelia, Chrysaora, and Cyanea in the lipid separation procedures outlined in earlier reports (Bligh-Dyer extraction and thin-layer chromatographic separations on Silica Gel G with hexane: ether: acetic acid, 80:20:1), a zone ("x") unique to Chrysaora polyps was observed. This zone, which is present in the medusae of the three species, was studied further.

The fatty acid components of "x" are the same as those of the triglyceride fraction. Although total characterization of this zone is still incomplete, its chromatographic properties and derivatives are consistent with those of alkoxy lipids (-alkyl glycerides, alkoxy alkanes).

This study was reported at the 6th Middle Atlantic Regional Meeting of the American Chemical Society (Joseph et al., 1971).

With very limited time on the gas-liquid chromatograph available to the jellyfish studies, a number of observations have already been made on the fatty acid constituents of neutral lipids:

1) The C_{18} and C_{16} fatty acids, both saturated and unsaturated, are quite prominent in the polyp triglycerides.

2) C_{20} and C_{22} fatty acids are present in polyp triglycerides.

3) The class "x" fatty acids (hypothesized as alkoxy diglycerides) have the same distribution of those of the triglycerides. The acylation of the "glycerol" thus involves a precursor pool of fatty acids which is common to that of the triglycerides.

4) Brine shrimp nauplii, the polyp food, have a fatty acid distribution significantly different from that of the polyps, particularly with respect to the longer chain fatty acids. Thus, the longer chain polyp fatty acids are either synthesized de novo or use C_{16} and C_{18} fatty acids of their food as the precursor for a chain elongation mechanism.

5) The fatty acids of the wild medusae triglycerides appear to contain a C_{17} fatty acid. This odd numbered fatty acid, although common in some estuarine and marine organisms (Ackman and Sipos, 1965) may serve as a useful guide in investigating organisms which serve as natural foods of the jellyfishes. Odd-numbered fatty acid distributions may

also aid in establishing the role that jelly-fishes play in the ecological balance of the estuarine habitat.

To date, the limited access and observation using gas-liquid chromatography for investigating the fatty acids of the neutral lipids are most promising. However, additional effort is necessary, employing this technique, for the complete characterization and quantitation of the fatty acids of the polyps and ephyrae.

2. Sub-Cellular Components

Table II shows the quantitation of representative nucleic acid analysis of Chrysaora. Average polyps were selected on 13 January while large ones were selected on 3 February. Although the sizes of the polyps differed (by visual observation at time of selection), the proportional amounts of nucleic acids (RNA/DNA ratio) is the same. This ratio is the same as that reported earlier by R. E. L. Black.

Other measurements are consistent with the "representative" non-strobilating Chrysaora polyp, consisting of 1 ug DNA, 10 ug RNA, 20-40 ug neutral lipid.

With these data on quantities of sub-cellular constituents and the approximate measurements made of single polyps, the design of biosynthetic experiments may proceed using assumptions of the "pool sizes" of precursor molecules which may be tested directly. In addition, the rates of synthesis may be estimated and tested with experiments designed to probe the mechanisms of setting and strobilation.

Table 1

Biochemical Assay Procedures

Substance	Reactants	Range	Reference
DNA	Diaminobenzoic Acid	0-10 ug	Kissane & Robbins, 1958
RNA	Orcinol - HCl-$FeCl_3$	0-20 ug	
Protein	Folin-phenol	0-150 ug	Lowry et al, 1951
Total Lipid	Sulfophosphovanillin	0-100 ug	Zollner & Kirsh, 1962
Cholesterol	o-Phthalaldehyde		Zlatkis & Zak, 1969

Table II

Nucleic Acid Content of Chrysaora quinquecirrha Polyps

Polyps Assayed	(1) 13 Jan '71	(10) 18 Jan '71	(8) 3 Feb '71
DNA	1.8 ± 0.5	0.5	5.3
RNA	19.2 ± 1.5	5.7	55.87
RNA/DNA	10.7	11.4	11.1

VI. References

Kissane, J. N. and Robbins, E. The Fluorometric Measurement of DNA in Animal Tissues. J. Biol. Chem. 233, 148-158 (1958).

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BIOCHEMICAL STUDIES OF STROBILATION

1. Metabolism of iodide

The distribution of iodide among several major classes of compounds was studied in polyps of Aurelia, by use of the radioactive isotope, I^{131}. Cold-conditioned and non-conditioned polyps, which had previously been grown in iodide free, artificial sea water at 20‰, were placed in water containing 1.2 micromoles per liter KI and 1.0 microcuries per ml I^{131}. Polyps were starved during the experiment. At intervals 100-200 polyps were removed and fractionated into acid-soluble, lipid, nucleic acid and protein fractions. Aliquots of each fraction were counted with a thin-window G-M tube and scaler. The results of this determination are shown in Table 1. The strobilating and non-strobilating polyps do not show any significant differences in iodide distribution among the classes of compounds studied. Even after a relatively short time, iodide is distributed throughout all classes of compound. Over a period of several days iodide percentages gradually increase in the lipid and protein fractions and decrease in the acid-soluble fraction.

The specific activity of the total protein was determined in conditioned and non-conditioned polyps, with the result presented in Figure 1. In both polyps the specific amount of protein-bound iodide increases at the same rate during the first several days. In the late strobila there is a decrease in specific amount bound; this probably indicates an increase in some protein which does not bind iodide.

It is concluded that the detection of unique iodide compounds formed prior to strobilation may be obscured by the general reactivity of iodide with so many different compounds in the polyp. The use of

extremely short pulse times, followed by chromatographic analysis of the acid-soluble fraction, might prove valuable.

2. Synthesis of DNA and RNA during strobilation

Synchronously strobilating Aurelia populations have been used to measure changes in levels of nucleic acids and protein during strobilation. In order to induce strobilation, polyps of uniform size, which had previously been held at 14-15° C for several months, were placed at 25° C and 1.2 micromoles KI per liter was added. Measurements of RNA, DNA and protein were made on: (1) strobilating polyps, (2) polyps from the same population in the absence of KI, (3) non-conditioned polyps in the presence of KI, and (4) non-conditioned polyps without KI. All polyps were starved during the entire period of measurement. The results are shown in Figs. 2, 3, and 4. DNA increases markedly in strobilating polyps, but in none of the non-strobilating ones. Increases in RNA and protein may also occur during strobilation but the changes are not large. Cold-conditioned polyps have lighter contents of RNA and protein than non-conditioned ones in the two populations measured. This is probably a result of decreased budding in the cold, but it may be related to the initiation of strobilation. Further information is needed about this. Iodide appears to have no effect on DNA, RNA or protein levels in non-conditioned polyps.

Autoradiographic analysis of DNA and RNA synthesis before and during strobilation have been continued, using polyps of Aurelia. When cold-conditioned polyps are warmed to 25°C and iodide is added, rapid DNA synthesis and cell division are observed within a few hours. The incorporation of thymidine-H^3 occurs throughout the epiderm, with the exception of the feeding tentacles, prior to strobilation. Few

or no cells in the gastroderm incorporate labeled thymidine during the pre-strobilation period. During mid- and late strobilation stages labeled cells are observed in the gastroderm of the developing ephyrae; however, these may have migrated inward from the epiderm. Labeled uridine (a precursor of RNA) is also incorporated at a high rate in the epiderm, but not in the gastroderm, prior to strobilation. RNA synthesis or turnover probably precedes strobilation; this is also indicated by inhibitor studies below.

A few inhibitors of RNA, DNA and protein synthesis have been used to attempt to block strobilation in polyps of Aurelia and Chrysaora. Chrysaora polyps were cultured in York River water at salinities of 19-22‰. Cold-conditioning was carried out at 12-14° C for at least 3 months prior to testing. Prolonged cold-treatment caused death of these polyps. Polyps were brought to 25° C 3 or 4 days before addition of inhibitor. Testing of inhibitors was done on batches of 50 polyps in 50 ml of water with inhibitor continuously present at 1 ppm or less (see below). Control batches of polyps were always taken from the same stock bowl as the experimental ones. Observation of the polyps was made daily for 30 days, until controls had ceased strobilating. The results of tests with Chrysaora polyps are shown in Table 2.

Table 1. Distribution of iodide among major classes of compounds in cold-conditioned and non-conditioned polyps. Columns (a) and (b) refer to separate batches of polyps.

Material	Hours in iodide		Total counts per minute		Percent in: Acid-Sol.		Lipid		Nucleic acid		Protein		Percent recovery	
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
Non-conditioned polyps	5	5	3,573	2,648	76.4	60.6	10.5	15.0	15.9	14.3	16.2	32.1	119	122
	17	17	4,799	8,211	64.6	28.0	7.9	9.1	12.4	10.7	20.1	34.0	105	82
	42	42	8,734	9,414	39.3	26.4	9.4	12.4	17.6	13.2	40.4	49.3	107	101
	65	65	18,945	13,248	32.6	46.2	8.1	12.5	14.2	16.4	36.7	53.2	92	128
		90		24,624		26.8		12.0		17.9		44.5		101
	117		25,533		26.2		7.7		16.9		29.1		80	
		163		30,789		17.6		12.0		14.9		54.8		99
Conditioned polyps	5	5	2,254	2,348	68.2	59.0	14.8	12.2	7.7	8.9	9.4	19.8	99	100
	17	17	8,424	4,433	68.5	52.2	18.2	14.0	11.2	12.7	14.9	39.6	113	119
		42		8,396		33.6		13.1		11.9		36.4		95
Early Strobila	42		36,621		50.4		18.4		5.8		9.4		84	
	65	65	60,660	16,587	47.3	42.1	16.1	15.5	7.0	11.3	10.5	27.0	81	96
Mid Strobila	90		40,725		51.3		15.8		13.5		14.9		96	
		117		69,417		31.2		15.4		11.6		34.8		94
Late Strobila	163	163	84,501	62,208	28.6	27.4	25.8	26.2	10.4	7.8	26.0	28.6	91	90

Table 2. Effects of inhibitors of synthetic pathways on strobilation in Chrysaora

Control polyps:

Batch	No. Strobilated	Ephyrae produced	Ephyrae/polyp
a	36	532	14.8
b	40	718	18.0
c	38	726	19.1
d	37	742	20.1
e	40	645	16.1
f	38	610	16.5

Inhibitors of DNA synthesis:

Inhibitor	Batch	Percent inhibition of ephyrae production	
5-Bromodeoxyuridine	a	0	
5-Iododeoxyuridine	a	0	
5-Bromocytidine deoxyriboside	b	57	
6-Azathymine	b	0	
5-Fluorouracil	b	83	(reversed on washing)
Ethidium bromide	c	0	
Nalidixic acid	c	0	

Inhibitors of RNA synthesis:

Actinomycin D	a	100	
8-Azaadenine	d	65	
8-Bromoadenosine	d	49	
8-Bromoguanosine	a	19	
6-Azauridine	b	40	
7 Deazaadenine (tubercidin)	e	100	(Polyps dead in 24 hrs.)
7 Deazaadenine (0.1 ppm)	f	70	
7-Deazaadenine (0.01 ppm)	f	0	
8-Aza-a-6-diaminopurine	d	0	
8-Azaguanine	b	0	
Rifampin	f	0	

Inhibitors of protein synthesis:

P-Fluorophenylalanine	a	78	(reversed on washing)
DL-m-Fluorotyrosine	f	100	(partially reversed on feeding)
3,5-Dibromotyrosine	f	0	

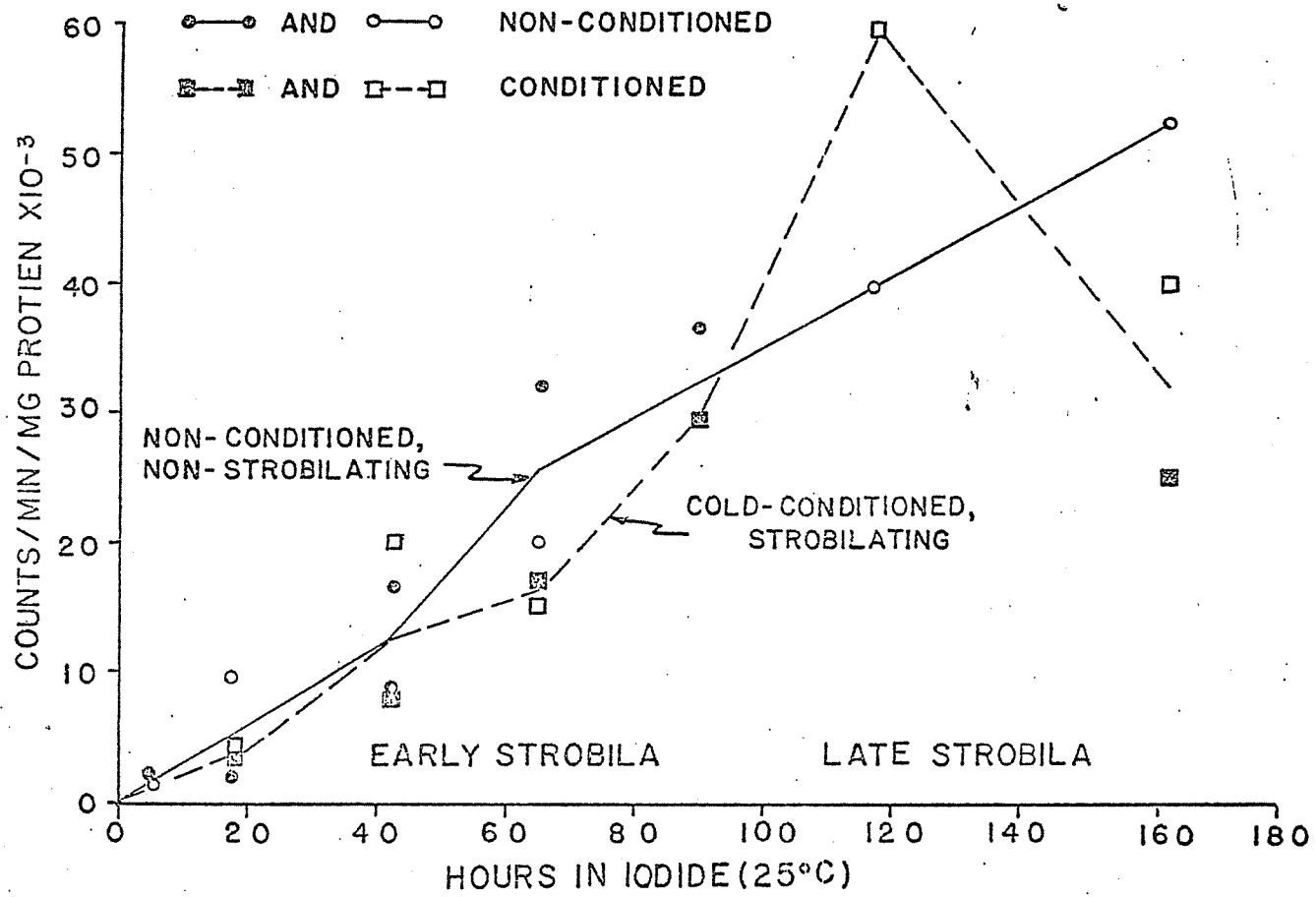

FIG. 1 - SPECIFIC ACTIVITY OF I^{131} - PROTEIN

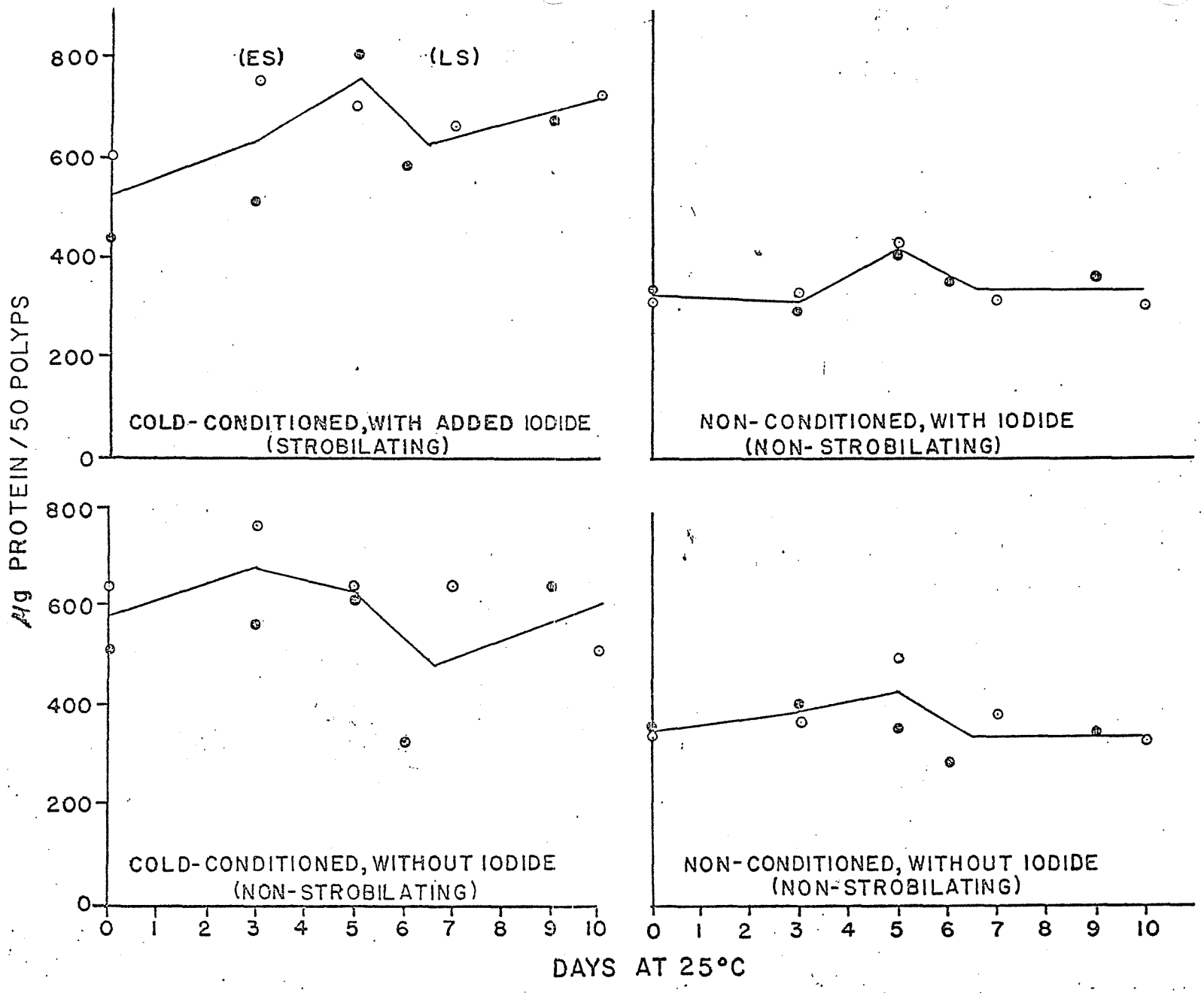
(ES)
(LS)
800
600
400
200
0
µg PROTEIN / 50 POLYPS
COLD-CONDITIONED, WITH ADDED IODIDE
(STROBILATING)
NON-CONDITIONED, WITH IODIDE
(NON-STROBILATING)
COLD-CONDITIONED, WITHOUT IODIDE
(NON-STROBILATING)
NON-CONDITIONED, WITHOUT IODIDE
(NON-STROBILATING)
0 1 2 3 4 5 6 7 8 9 10
DAYS AT 25°C

FIG. 2 - DNA

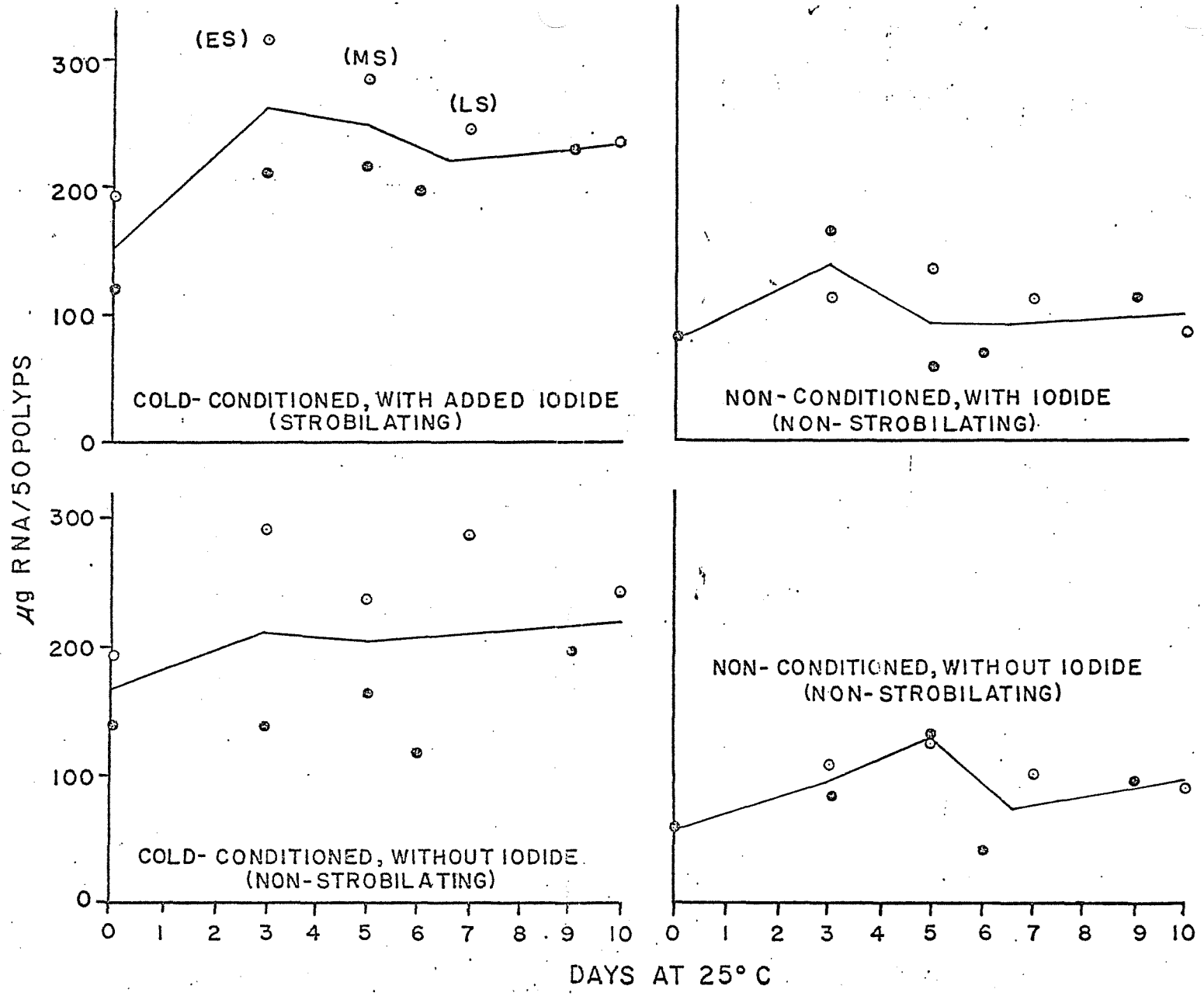
(ES)
(MS)
(LS)
300
200
100
0
μg RNA/50 POLYPS
COLD-CONDITIONED, WITH ADDED IODIDE
(STROBILATING)
NON-CONDITIONED, WITH IODIDE
(NON-STROBILATING)
COLD-CONDITIONED, WITHOUT IODIDE
(NON-STROBILATING)
NON-CONDITIONED, WITHOUT IODIDE
(NON-STROBILATING)
0 1 2 3 4 5 6 7 8 9 10
DAYS AT 25° C

FIG. 3. RNA

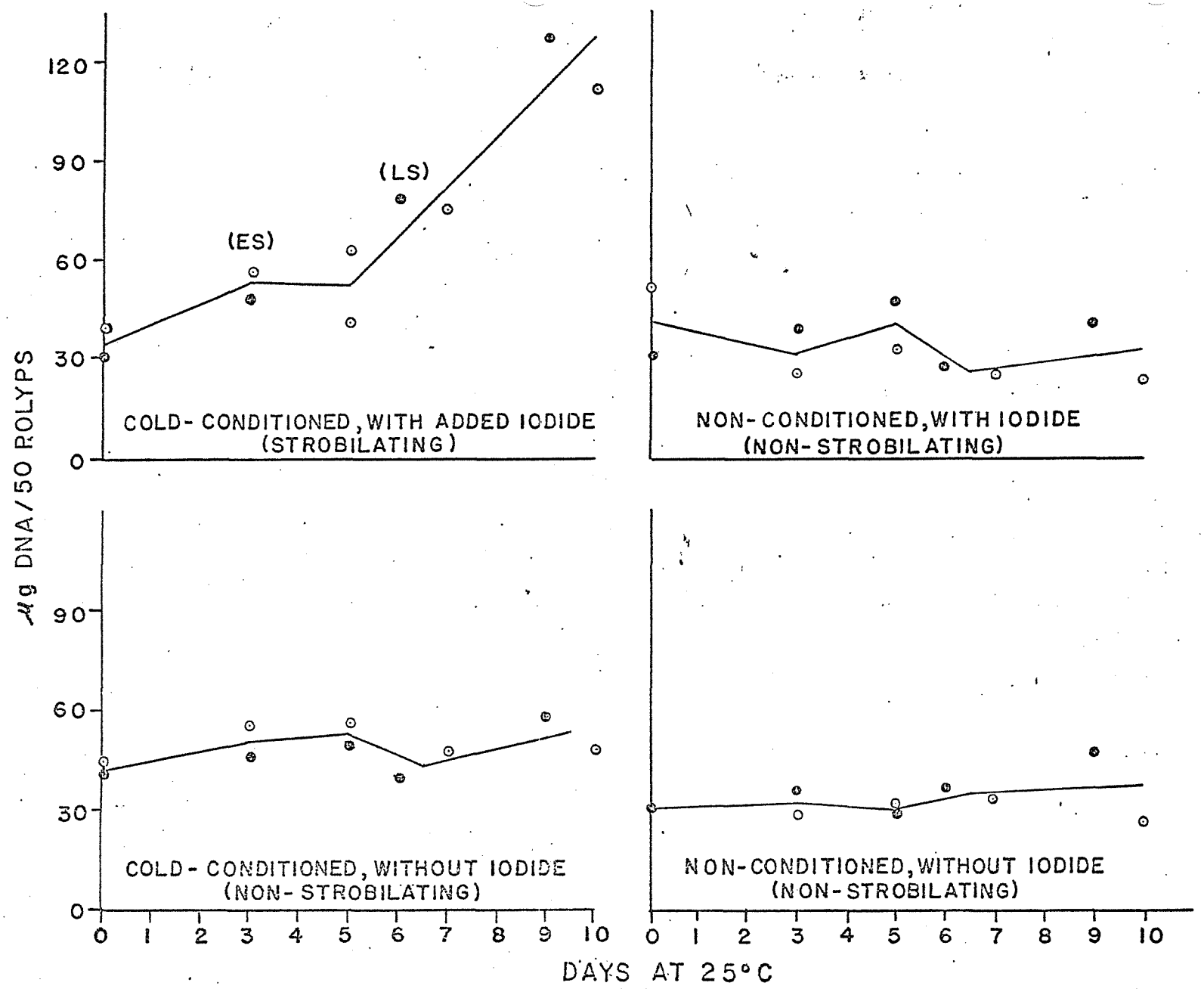

FIG. 4 - PROTEIN

JELLYFISH PHYSIOLOGY

INTRODUCTION

During the period covered by this report a number of physiological studies have been undertaken. These studies may be roughly broken up into three categories:

1) The phenomenon of planula setting and metamorphosis.
2) Measurement of metabolism as reflected by respiration.
3) The role of amino acids and their relationship to the osmoregulatory mechanisms.

As in so many cases of experimentation in which an organism is involved, biological variability enters into the experimental design. Quite often in biological studies the variability within the biological population is such that clearly defined results in answering the questions posed are not always obtainable. In the studies which follow, rather specific questions were asked within the design of the various experiments. In most cases where quantitative information was desired, enough data was obtained for generalized conclusions although some of the specific questions originally posed remain enigmatic.

Although the major emphasis of the program on jellyfish control is directed to the interruption of the strobilation phase of the life cycle, experiments devoted entirely to the mechanisms of strobilation in Chrysaora are difficult at this time because precise laboratory conditions for inducing strobilation are still unknown. Refinements of experiments involving the manipulation

of salinity and temperature conditions are still underway. Some early results of these experiments are reported here.

I. PLANULA SETTING

An interruption of any phase of the jellyfish life cycle is a strong possibility for the introduction of a control mechanism. The phenomenon of planulae setting has been investigated. If an inhibitor of setting is uncovered, the formation of polyps will be prevented because either the larval cyst or the attached planulae stage will be prevented. The planulae setting experiments fall into 2-sub-groupings: those with Cyanea capillata and others with Chrysaora quinquecirrha.

A. Cyanea capillata

After fertilization in Cyanea capillata occurs, the planulae develop from the fertilized egg and remain attached to the oral arms. The ciliated planulae may be liberated from the oral arms. The metamorphosis from the free swimming planulae to the larval cyst then occurs. In these experiments the temperatures and salinities were varied.

Under the experimental conditions employed, the effects of temperature and salinity were ascertained. At least 80% of the planulae set within 72 hours at temperatues of 10, 15 and 20°C and at the salinities covering the range of 5 to 45 parts per thousand. However, of the limited number of polyps that developed from the larval cysts, the salinity range was restricted to 15 to 35 parts per thousand.

B. Chrysaora quinquecirrha

In contrast to Cyanea capillata, which has planula attached to the oral arms, the Chrysaora planulae are liberated and

free swimming. The Chrysaora planulae attach to a substrate directly in the setting process. They do not form larval cysts as in the case of Cyanea.

Observations on planulae setting with Chrysaora were studied with the addition to the media of lampreycides in concentrations ranging from 0.1 to 2 parts per million. In general, the 3 lampreycides which inhibit Hydra also inhibited the number of planulae which set. However, at the concentrations employed, undoubtedly larvae of desirable species would also be destroyed.

II. RESPIRATION STUDIES

Respiration studies were undertaken with Chrysaora polyps using both the Warburg respirameter and the polarographic oxygen probe. With the Warburg usually 10 polyps were needed per vessel. Measurements of respiration were obtained over periods of 1/2 to 3 hours at various temperatures. In view of the large number of polyps which were necessary for the respiration studies and the long periods of time needed for these studies, the alternative using the oxygen probe was pursued. It was desired to make measurements on single polyps over shorter periods of time (i.e., 5-15 minutes). Using both these techniques, measurement of oxygen uptake usually ranged in the order of .5 to 4 ul of oxygen/hour/polyp. With this large variation in measurements of the small amounts of oxygen uptake per hour, detailed studies were not only difficult to perform but also difficult to interpretate. Variations with additions did not become apparent due to the small changes in oxygen measurement which were observed. Effects of additions or experimental conditions

were usually less than 20% of the initial conditions, an arbitrarily established limit which would permit unequivocal interpretations. It is still quite probable that with scale expansion on the recorder and with a chamber modified for polyps specifically, that the polarographic technique may still be applicable to metabolic studies.

III. FREE AMINO ACID COMPOSITION OF SCYPHOZOAN POLYPS AT VARIOUS SALINITIES

As part of a continuing study of the scyphozoan jellyfish of Chesapeake Bay it was decided to investigate the free amino acid (FAA) pools of these organisms. Essentially very little information is available in the literature concerning the identification of or pool size of individual FAA in the Cnidaria or Coelenterata or closely related groups. von Holt (1968) indicates that 35% of the FAA of coral tissue from Zoanthus flos marinus is glycine of a total of 10 uM/mg protein N.

Lane et al (1965) have published single analyses of what appear to be a sum of free and comined (protein) amino acids of Aurelia and Physalia extracellular fluids. Glycine, the most concentrated individual FAA in Aurelia fluids, comprised 15.8% of the total of 6610 uM/ml. The most abundant FAA in many marine invertebrates is glycine (see Awapara, 1962).

Sample preparation:

Unless otherwise stated polyps were harvested 48 hours after feeding. Cysts were removed 2 days before harvesting to allow regeneration time. Polyps were handled by pipet after removing any visible extranous material with dissecting tools, placed in a 15 ml graduated centrifuge tube and raised with artificial sea

water (ASW) of appropriate salinity which was newly filtered through a 0.45 u membrane filter and as much water removed as convenient. A simultaneous amino acid extraction-protein precipitation was carried out by adding 4 volumes of absolute ethanol and if necessary enough additional 80% ethanol to make a convenient working volume; the sample was immediately sonicated and then centrifuged at 2000 g, 10° C for 10 minutes. The residue was reextracted with 80% ethanol by suspending the pellet by sonicating and recentrifuging; the combined extracts as well as the residue were stored at freezer temperature prior to analysis.

Analytical procedures:

Amino acid analysis was performed on an aliquot of stored extracts to which an internal standard of 0.25 uM norleucine was added; mixture was dried under reduced pressure at 40 C, redissolved in 0.1 N HCl and injected under N_2 pressure into a 0.6 X 129 cm chromatographic column of Tehnicon Chromobeads B. Elution was carried out at 60 C with a gradient of sodium citrate buffers (pH 2.875-5.00) at a flow rate of 0.5 ml/min. Quantitation was carried out by reaction with ninhydrin and measurement of color development at 440 and 570 mu through 15 mm flow cuvettes. Preliminary identification was based on Rf values, combined sample-chromatography of material obtained by use of a fraction collector on a sample splitter between the chromatographic column and the ninhydrin.

Protein was determined by the method of Lowry (Lowry, et al (1951)) using 0.1 N N_{aOH} to dissolve an aliquote of protein precipitate.

DNA was measured on an additional aliquote of the protein precipitate by the method of Holm-Hansen et al (1968).

Concentration of FAA in Aurelia aurita and Chrysaora quinquecirrha lab polyps starved 48 hours is related to salinity (Tables 1,2).

With Chrysaora quinquecirrha a major shift in relative mole % occurs between 10 and 15% with similar proportions from 15-30. Glycine is doing all the work or changing in quantity.

Aurelia aurita also makes a major shift between 10 and 15 in FAA proportions (big drop in Glut. A, Lysine). Increases are obvious in glycine, B-alanine and taurine, unlike Chrysaora quinquecirrha which is glycine alone.

Chrysaora quinquecirrha field polyps which were starved for 48 hours show the same patterns as the starved laboratory Chrysaora polyps at the comparable salinity although the concentration (NM/mg protein) are the lowest measured in any polyps. The Chrysaora quinquecirrha field polyps sampled immediately after collecting showed great variation in both relative composition and concentration although they were from a fairly narrow salinity range (13-16‰). Relative 14 high concentrations of taurine and alanine in F-1 undoubtedly reflect the food upon which the polyps had been feeding as do the other great differences in proportions and total concentrations. F-4, although sampled after collection shows the same proportions and total as 48 hr starved polyps and may be assumed to have had low food availability at that time. Aurelia polyps obtained from MBL and maintained in the lab show an FAA picture closer to Chrysaora quinquecirrha than to the Virginia

Aurelia aurita polyps. They have high glycine and little B-alanine.

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